

1 **Phase 1 clinical study of an embryonic stem cell–derived retinal pigment
2 epithelium patch in age-related macular degeneration**

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27

28 **ABSTRACT**

29 **Age-related macular degeneration (AMD) remains a major cause of blindness, with**
30 **dysfunction and loss of retinal pigment epithelium (RPE) central to disease**
31 **progression. We engineered an RPE patch comprising a fully differentiated, human**
32 **embryonic stem cell (hESC)-derived RPE monolayer on a coated, synthetic**
33 **basement membrane. We delivered the patch, using a purpose-designed**
34 **microsurgical tool, into the subretinal space of one eye in each of two patients with**
35 **severe exudative AMD. Primary endpoints were incidence and severity of adverse**
36 **events and proportion of subjects with improved best-corrected visual acuity of 15**
37 **letters or more. We report successful delivery and survival of the RPE patch by**
38 **biomicroscopy and optical [AU: OK?] coherence tomography, and a visual acuity**
39 **gain of 29 and 21 letters in the two patients, respectively, over 12 months. Only local**
40 **immunosuppression was used long-term. We also present preclinical, surgical, cell**
41 **safety and tumorigenicity studies leading to trial approval. This work supports the**
42 **feasibility and safety of hESC-RPE patch transplantation as a regenerative strategy**
43 **for AMD.**

44

45 **INTRODUCTION**

46 Human ESCs represent a promising source for cellular replacement therapies owing to
47 their availability, pluripotency, and unlimited self-renewal capacity. However, they also
48 carry risks of neoplastic change, uncontrolled proliferation, and differentiation to
49 inappropriate cell types^{1, 2}. The eye is advantageous in investigating hESC-based cell
50 therapy, as it is accessible and confined, and the transplanted cells can be monitored
51 directly *in vivo*, with the possibility of being removed or destroyed if there is evidence of
52 neoplastic change^{3, 4}. Furthermore, long-term immunosuppression can be delivered locally.

53

54 Late AMD is characterized by irreversible cell loss, initially of RPE cells and subsequently
55 of neuroretinal and choroidal cells⁵, and thus may be amenable to hESC-based cell
56 therapy⁴. The disease process includes damage to the RPE's specialized basement
57 membrane, Bruch's membrane⁵. Currently, treatments exist only for the exudative or 'wet'
58 form of AMD. These treatments rely on angiogenesis inhibitors⁶ or indirect transplantation
59 of an autologous RPE-Bruch's complex (retinal translocation surgery)⁷.

60

61

62 However, the former treatment only suppresses the disease, requiring long-term
63 repeat delivery, and the latter, although restoring the macular anatomy, does not prevent
64 disease recurrence. It is possible that hESC-RPE patch may alter the natural history of the
65 disease as the cells are zero years old, rather than the 60 plus years of the patients, and it
66 does not contain predisposition to develop AMD that the host cells have already manifest,
67 however, only further investigations will address this. There is no treatment for atrophic
68 'dry' AMD, which is characterized by RPE loss and progressive neuroretinal cellular
69 dysfunction.

70 Suspensions of hESC-derived RPE (hESC-RPE) cells have been transplanted in
71 human subjects with dry AMD and Stargardt's disease, but the extent of cell survival and
72 restoration of vision remains ambiguous ⁸. A recent, single-patient report described
73 transplantation of an autologous induced pluripotent stem cell (iPSC)-derived RPE patch
74 on its own secreted basement membrane²⁹. The iPSC-RPE survived with maintenance, but
75 no improvement, of visual acuity at 12 months. We developed a therapeutic, biocompatible
76 hESC-RPE monolayer on a coated synthetic membrane, herein termed a 'patch', for
77 transplantation in wet and early-stage dry AMD. The choice of membrane material and its
78 preparation, including the human vitronectin coating, has not been described previously to
79 our knowledge. In contrast to RPE suspensions, cells on the patch are delivered fully
80 differentiated, polarized, and with the tight junction barrier formed, that is, in a form close to
81 their native configuration. The synthetic membrane allows the patch to be handled easily
82 and robustly. The main disadvantage of the patch is that it requires a purpose-built delivery
83 tool and a more complicated surgery compared to cell suspensions, and the use of hESCs
84 may require immunosuppression, unlike an autologous cell source. Our delivery tool
85 (Supplementary figure 1) confers the benefit of protecting the patch within the tip, thereby
86 minimizing cell loss, cell distribution within the eye, and physical damage to the RPE
87 monolayer.

88
89 The clinical trial was designed as a phase 1, open-label, safety and feasibility study
90 of implantation of a hESC-RPE patch in subjects with acute wet AMD and recent rapid
91 vision decline. For safety reasons and to obtain an early efficacy signal, the initial clinical
92 trial involved patients with severe wet AMD only, although we aim to study the RPE patch
93 in early dry AMD as well. We reported three serious adverse events to the regulator. These
94 were exposure of the suture of the fluocinolone implant used for immunosuppression, a
95 retinal detachment, and worsening of diabetes following oral prednisolone. All three

96 incidents required readmission to the hospital, with the first two incidents requiring further
97 surgery and the third being treated medically. The three incidents were treated
98 successfully. Both patients achieved an improvement in vision of more than 15 letters,
99 which was sustained for 12 months.

100

101 RESULTS

102 Engineering an RPE patch

103 Our protocol for manufacturing a clinical-grade RPE patch is summarized in **Figure 1**. RPE
104 cells were differentiated from the SHEF-1.3 hESC line, which had been derived from the
105 SHEF1 hESC line. We used a spontaneous differentiation method ⁹, an approach that has
106 been described earlier by others and has been applied to primate ¹⁰ and human ^{11, 12}
107 ESCs. Spontaneous differentiation limits the need for additional factors, thus complying
108 with good manufacturing practice (GMP) guidelines, and with clear advantages over
109 vector-driven methods in terms of safety and stability of the final RPE. Our differentiation
110 protocol differs from another similar protocol ⁹ in that we used Essential 8 medium rather
111 than human feeder cells during expansion and the early phase of differentiation to avoid
112 exposure to another cell type and to fulfill GMP guidance. While several methods are used
113 to produce hESC-RPE ^{10, 12, 13, 14}, because of epigenetic variation from the earliest stages
114 after derivation of hESCs, no method is universally effective ^{14, 15}. With our protocol, 20–
115 30% of hESC colonies differentiated to RPE (data not shown), which is similar to that of
116 other spontaneous methods ^{10, 12} but less efficient than some augmented methods ^{14–16}.

117

118 Subsequent RPE characterization used immunocytochemistry, electron microscopy,
119 pigment-epithelium-derived factor (PEDF) secretion profile testing, and a functional
120 phagocytosis assay (**Fig. 2d**). During manufacturing, in-process testing, including *in situ*
121 hybridization with a specific oligonucleotide probe for LIN28A mRNA¹¹, was undertaken to
122 detect hESC impurity at the single-cell level. Differentiated RPE was discarded if any
123 LIN28A-positive cells were detected. The hESC-RPE cells were seeded at confluence onto
124 a human-vitronectin-coated polyester membrane. A 6 × 3 mm (17 mm²) therapeutic
125 element with ~100,000 cells (PF-05206388 in the regulatory documentation) was cut to
126 size with a purpose-built punch and loaded into a sealed transport container (Fig. 1).
127 Immediately prior to release for human transplantation, the cell layer was recharacterized
128 as RPE according to a visual inspection release test encompassing cell dose, cell identity,
129 and patch coverage checks.

130

131 **Mouse teratoma and *in vitro* cell-spiking studies**

132 The literature suggests that adult human RPE cells¹⁷, terminally differentiated hESC-
133 RPE¹⁸, and spontaneously immortalized RPE cells¹⁹ are non-proliferative cell types and
134 appear to lack the potential to form teratomas. We tested tumorigenicity in NIH III nude
135 mice. Cell suspensions were used as the mouse eye is too small to administer a patch.
136 Two initial studies conducted with undifferentiated hESCs showed that teratomas could
137 form. In these studies, cell suspensions were injected subretinally, intra-muscularly and
138 subcutaneously, with mice followed for 26 weeks. Subsequently, we studied the
139 tumorigenicity of hESC-RPE cells under good laboratory practice (GLP) conditions,
140 including a positive control group injected with undifferentiated hESCs. Given our finding
141 that undifferentiated hESCs formed teratomas, we conducted *in vitro* cell-spiking studies to
142 examine whether hESCs survive the RPE manufacturing process and culture-seeding
143 conditions.

144

145 In the first study, injection of $4.5\text{--}8.8 \times 10^4$ undifferentiated hESCs into the subretinal
146 space of NIH III mice resulted in localized neoplastic formation in almost half the males
147 injected (12/30) but in very few females (2/30). The tumors showed evidence of
148 pluripotency and appeared to be composed of mesenchymal and epithelial lineages (data
149 not shown).

150

151 In the second study of undifferentiated hESCs, neoplastic masses were observed in
152 the injected eye of 12/15 female and 5/5 male mice after cell administration into the
153 subretinal space. Teratomas were observed microscopically in the thigh of all female mice
154 that received 3.6×10^4 or 8.23×10^5 undifferentiated hESCs in BD Matrigel by the
155 intramuscular route, and in the left flank of 2/5 and 1/11 female mice that received $3.6 \times$
156 10^4 or 8.23×10^5 undifferentiated hESCs in BD Matrigel, respectively, by the subcutaneous
157 route. Masses were composed of structures derived from all embryonic germ layers (data
158 not shown).

159

160 In the third study, conducted with cells manufactured under GLP conditions,
161 suspensions of hESC-RPE cells, or of undifferentiated hESCs as a positive tumorigenic
162 control, were injected subretinally in NIH III mice. In a total of 80 mice injected with
163 suspensions of hESC-RPE cells, no teratomas were detected (Fig 3B). Pigmented hESC-

164 RPE cells were observed lining the surface of the retina or lens in the injected eyes of
165 some mice given 6.04×10^4 hESC-RPE cells (Fig.3 E1, E2 and E3 at 26 weeks (Fig.3E1).
166 No premature death was associated with the injections.

167

168 In the same study, administration of undifferentiated hESCs was associated with
169 ocular teratoma formation in four males and five females given 4.28×10^4 cells, all of which
170 were prematurely euthanized (between days 46–62), and in a single male given 4.51×10^3
171 cells, which survived to the 26 week end point of the experiments (Fig 3C). In addition,
172 mesenchymal tumors classified as “Not Otherwise Specified” were present in two males
173 given 4.28×10^4 undifferentiated hESCs (Fig 3D) and perilenticular mesenchymal
174 hyperplasia in animals of both sexes given 4.51×10^3 cells or 4.28×10^4 cells. All tumors
175 were composed of human cells, as confirmed by immunohistochemistry using an anti-
176 human mitochondrial marker (data not shown). Eyes injected with undifferentiated hESCs
177 also exhibited an increase in the incidence and severity of non-proliferative changes,
178 including lenticular degeneration, posterior synechiae of the iris, and retinal detachment
179 (data not shown). Transplantation of either differentiated or undifferentiated hESCs did not
180 affect body weight.

181

182 Given the tumor formation observed with undifferentiated hESCs, we aimed to
183 determine whether hESC-RPE derived from SHEF-1.3 hESC contained any residual
184 pluripotent cells or could support the survival of pluripotent cells, as this would constitute a
185 teratoma risk. Immunostaining and flow cytometric analysis of cells expressing the
186 pluripotency marker Tra-1-60 (Abcam 16288, Cambridge, UK) detected no undifferentiated
187 hESCs in dissociated RPE foci prior to the expansion phase or at 6 to 8 weeks after
188 seeding at the end of the expansion phase (Supplementary figure 2). We spiked
189 undifferentiated hESCs into the RPE at 1, 10, 20 or 50% of total cells at the start of the
190 expansion phase (see online methods). This did not affect the quality of the resulting 6-to-
191 8-week-old cultures at the end of the expansion phase, which showed cobblestone
192 morphology and pigmentation, and stained strongly for PMEL17 (an RPE marker that
193 indicates the presence of premelanosomes). By 2 d post-spiking, viable Tra-1-60-positive
194 cells were no longer detectable by flow cytometry (data not shown). This finding was
195 supported by propidium iodide staining, which showed that ~96% of hESCs died when
196 dissociated and seeded in the same manner as RPE prepared for expansion
197 (Supplementary figure 3). The cells that survived in some experiments did not have hESC-

198 like morphology and failed to stain for Tra-1-60 or the proliferation marker Ki67, suggesting
199 that they had differentiated or senesced (Supplementary figure 3 D&E).

200

201 **Pig single-dose studies**

202 Studies of surgical feasibility and safety of delivery of the RPE patch as well as studies of
203 local and systemic biodistribution and toxicity were carried out in pigs. Human and pig eyes
204 are similar in size, which allowed for administration of the full-size patch. Two studies were
205 performed; the second used the same clinical surgical technique as in the human clinical
206 trial. Biodistribution of hESC-RPE cells was evaluated in more than ten sites using qPCR
207 detection of multiple human cell markers.

208

209 In the first pig study, patch delivery was successful in all 20 animals, as assessed by
210 intraoperative observation of the patch under the neural retina and histological sections
211 showing the patch in the subretinal space (figure 3 H & I). No retinal detachments were
212 noted in any animal. We found surviving human cells by light microscopy at 2 h, 2 d, and 1,
213 2, 4, and 6 weeks after implantation (RPE patch: $n = 12$; control patch without cells: $n = 8$;
214 figure 3 H-K). Human [AU: OK?] cell survival was demonstrated despite
215 immunosuppression having been limited to the peri-operative period using oral
216 prednisolone. Surviving cells remained pigmented (Fig. 3 J), expressed RPE-specific cell
217 markers, showed no proliferation activity, and did not migrate away from the membrane
218 (data not shown). [AU: Clarify: 'Human' or 'Human or pig'?] Pig photoreceptor survival
219 above the patch was observed only in animals that received an RPE patch, as assessed by
220 histology (Fig. 3H). The control membrane without cells did not pig photoreceptor survival
221 (Fig. 3I). Microscopically, retinal architecture was intact in most animals receiving human
222 cells. Macrophages were seen and appeared to have phagocytosed some transplanted
223 RPE cells to form large pigmented cells (data not shown). Lymphocytic infiltrate was not
224 observed except in 2 animals at 2 and 6 weeks. Animals that received the control
225 membrane also had macrophage infiltrates but no lymphocytic response (data not shown).
226 These results demonstrated that patch transplantation with hESC-RPE survival was
227 surgically feasible without significant safety issues.

228

229 The second pig study differed from the first in that we used the purpose-built
230 surgical tool, which has a protective cradle at the tip from which the patch is mechanically
231 pushed out when the tip is in the subretinal space (Supplementary figure 1). The study was

232 undertaken under GLP conditions, a standardized set of requirements to ensure quality
233 and systematic management of the experiment. In this case, it dictated standards for the
234 operating and animal facilities and for the personnel who ran the trial, and defined the
235 conditions and requirements for the keeping and monitoring of animals in the study,
236 including the feeding regimen. Correct surgical delivery of ten RPE patches and ten coated
237 membranes without RPE was achieved in 20 pigs as assessed by clinical examination and
238 histology (Figure 3 F & G). Good cell cover was observed at the 6-week time point in the
239 ten animals that received RPE cells (Fig. 3F). Retinal detachment and rupture of the lens
240 capsule were observed in one male and one female implanted with the patch. There was
241 no evidence of weight loss or early death in any animal. At 6 months after implantation, no
242 hESC-RPE cells were detected at the implantation site or elsewhere in H&E sections of the
243 ten eyes receiving the RPE patch. Anti-human TRA-1-85 immunostaining of the operated
244 eye and optic nerve from these animals was also negative. Microscopic findings of chronic
245 inflammation were seen restricted to the subretinal implantation site, at 6 months in the
246 implanted (left) eye of all animals, and were consistent with the intraocular surgical
247 implantation procedure. The microscopic findings included fibrosis, osseous metaplasia,
248 and small numbers of macrophages and multinucleate giant cells (data not shown).
249 Atrophy of the photoreceptor layer in overlying retina was also present (data not shown).
250 Findings were of similar incidence and severity in both control and cell-treated animals. No
251 positive amplification of any human-specific genes was observed in adrenal, bone marrow
252 (rib and femur), brain, heart, kidneys, liver, lungs, lymph nodes, optic nerve, spleen, or
253 thymus, which were evaluated by qPCR, indicating that the cells did not appear to migrate
254 or survive away from the site of implantation. The second pig study confirmed the surgical
255 feasibility and reliability of the delivery tool and the lack of systemic or local distribution of
256 the hESC-RPE cells.

257

258 **Clinical trial**

259 Regulatory permission was granted for a phase I trial on the basis of the preclinical studies
260 reported in this article, and published data on the hESC-RPE monolayer⁹ (Clinical
261 Trials.gov: NCT01691261). Permission was granted for ten patients, and we report the
262 primary and secondary outcomes from the first two (Figs 4, 5, 6 and 7). In addition to
263 safety, the trial investigated whether the synthetic membrane would facilitate mechanical
264 delivery of the RPE monolayer, whether the transplanted cells could be sustained long-

265 term with local immunosuppression only, and whether early signals of potential efficacy
266 were evident.

267

268 Using the surgical delivery tool, we placed one RPE patch in the subretinal space,
269 under the fovea, in the affected eye of each patient. Correct placement was confirmed in
270 both patients by stereo-biomicroscopy, fundus photography, and spectral domain optical
271 coherence tomography (SD-OCT) (Figs. 4b1, b2 and 5b1, b2).

272

273 In patient 1, OCT and native-level autofluorescence immediately after surgery
274 showed a 'double thickness' of RPE in one area nasal to the fovea, indicating overlap of
275 native RPE and the patch (Figs. 4b, c, d). In all other areas of patient 1, and in all areas of
276 patient 2, a single layer of RPE on OCT suggested there was no residual native RPE over
277 the patch (figures 4b1 and 5b1). In both patients, hESC-RPE remained present over the
278 full area of the patch at 12 months as evidenced by dark pigmented cells covering the
279 patch, although unevenly, on fundus photography and a hyper-reflective monolayer on the
280 patch seen by SD-OCT (figures 4a1,b1, 5a1, b1 and 6a and b). In both patients, the
281 patches showed uneven autofluorescence (figures 4e3 and 5e3), which suggests
282 functioning RPE phagocytosis^{20,21}. Also, visible in both patients were darker, pigmented
283 areas continuous with the patch, which may represent RPE cell migration off the patch onto
284 adjacent RPE-deficient areas. These areas spread from the patch edge outward over the
285 first 6 months after surgery before stabilization (Fig. 6 a and b). The areas were contiguous
286 with the patch RPE signal on OCT and were absent in areas where the native RPE layer
287 persisted. There was no evidence of neoplastic transformation either on regular review of
288 the fundus by an ocular oncologist or by serial ocular ultrasound.

289

290 In addition to RPE survival, we studied visual recovery. All measurements of visual
291 acuity were made once before surgery and once at 12 months after surgery, and testing
292 was always carried out by an independent qualified observer. The Early Treatment
293 Diabetic Retinopathy Study (ETDRS) letter chart was used to define best corrected visual
294 acuity (BCVA—vision with an optometrist-determined best glasses-corrected vision), which
295 improved from 10 to 39 and from 8 to 29 letters, over 12 months in patients 1 and 2,
296 respectively (Figs 7a and 7c). Microperimetry, a test of perception of microlocalized light
297 stimuli, showed visual fixation at the center of the patch and vision over the patch in both
298 patients (Figs. 4b1 and 5b1). Figures 4b1 and 5b1 show sample areas of visual sensitivity

299 localized totally within the patch. Over 12 months, reading speed improved from 1.7 to 82.8
300 and from 0 to 47.8 words/min in patients 1 and 2, respectively, by Minnesota MN Read
301 (figs 7b and 7d), an improvement and final level not found in the Submacular Surgery Trial
302 ²². Pelli-Robson contrast sensitivity scores (Log) improved from 0.45 to 1.35 in patient 1,
303 and 0 to 1.05 in patient 2, over 12 months. At each point that showed microperimetry
304 sensitivity over hESC-RPE, we observed choroidal filling by angiography (Figs. 4e1, 4e2,
305 5e1 and 5e2); RPE-autofluorescence (Figs. 4e3 and 5e3; and presence of the ellipsoid
306 layer (indicative of preserved photoreceptors) by SD-OCT (Figs. 4c1, 4c2, 5c1 and 5c2).
307 We note that it was not possible to ascertain whether the ellipsoid zone was present pre-
308 operatively, owing to the poor detail in the pre-operative OCT scans (Figs. 4a2 and 5a2).
309 Rtx1 Adaptive optics camera images showed survival of cone photoreceptors in the areas
310 corresponding to areas of sensitivity on microperimetry (Figures 4d1, 4d2, 5d1 and 5d2).
311

312 We reported three serious adverse events that were unrelated to the RPE patch.
313 The first was exposure of the suture of the fluocinolone implant in patient 1, which required
314 conjunctival revision surgery. The other two, both in patient 2, were a worsening of
315 diabetes following oral prednisolone, which was treated medically, and a retinal
316 detachment. The retinal detachment was an asymptomatic, infero-temporal, proliferative
317 vitreoretinopathy (PVR)-associated traction retinal detachment under silicone, which did
318 not extend past the inferior arcade and thus did not affect the implant. It was observed at
319 the 8-week follow-up, with the retina having been completely attached at the 4-week check.
320 It was treated with a single surgery with peeling of the PVR membranes, inferior
321 retinectomy of the peripheral retina (180 degrees), and laser to the retinectomy edge. The
322 silicone oil was retained. The retina was attached at the end of the surgery and has
323 remained attached after subsequent surgery to remove the silicone oil. There was a
324 residual epiretinal band over the posterior pole with some focal macular traction (figures
325 5b1 and 6b.), which was not treated.
326

327 Ocular pressures were never raised in either patient. No changes of concern were
328 noted in the liver and renal function tests and by liver ultrasound in either patient. On full-
329 field electro-retinography (ERG) recording, there was evidence of a mild but consistent
330 reduction in photoreceptor function at 6 months in both patients with additional consequent
331 Electro-oculography (EOG) reduction (in the operated eyes). The reduction in
332 photoreceptor function persisted in patient 1 but recovered in patient 2 by 12 months.

333

334 Discussion

335 The results presented here provide an early indication of the safety and feasibility of
336 manufacturing an hESC-RPE monolayer on a synthetic basement membrane and
337 delivering the patch into the subretinal space as a potential treatment for AMD. Our data
338 suggest early efficacy, stability, and safety of the RPE patch for up to 12 months in two
339 patients with severe vision loss from very severe wet AMD.

340

341 hESC-RPE on a membrane shows optimized differentiation, polarization, viability,
342 and maturation of the monolayer at the time of delivery that contrasts favorably with
343 delivery of cell suspensions, where the cells are necessarily not in a monolayer and
344 thereby not polarized or fully differentiated. Proper orientation is readily confirmed by the
345 color difference between the white membrane and the pigmented RPE. Cells delivered in
346 suspension may be lost due to reflux through the retinotomy, with potential vitreous
347 seeding, and the cells undergo shear stress and damage when ejected through the
348 delivery cannula²³. Furthermore, cells in suspension are required to adhere to and form a
349 monolayer on a damaged native Bruch's membrane, which leads to poorer cell survival
350 and widespread apoptosis²⁴. Previous work showed poor differentiation using suspensions
351 and that RPE monolayers on membranes appear superior²⁵.

352

353 Therapeutic human RPE patch transplantation has been reported using a harvested
354 autologous RPE–Bruch's membrane–choroid patch from the same eye^{26, 27, 28} and an
355 induced pluripotent stem cell (iPSC)-derived RPE patch²⁹. The main advantages of our
356 system over these were mechanical ease of handling due to the rigidity of the synthetic
357 membrane. The tool we developed also allowed consistent patch delivery with a small
358 localized retinal detachment over the macula, whereas with the intraoperative harvested
359 autologous technique, half of the entire retina must be reflected to ensure consistent
360 delivery without RPE damage^{30, 31}. The availability of an off-the-shelf patch is especially
361 advantageous and critical in cases of severe wet AMD with sudden vision loss, as
362 described in this study. Treatment is required rapidly³², and an autologous iPSC patch
363 could not be prepared in a suitable time frame for transplantation. The main disadvantage
364 of our technology is the need for immunosuppression, although for the two cases reported
365 here we have demonstrated that only local immunosuppression is necessary for long-term
366 hESC-RPE survival.

367

368 We present key preclinical safety and tumorigenicity studies that supported
369 regulatory approval of our clinical study. The GLP study of tumorigenicity in NIH III mice
370 showed that the hESC-RPE was not associated with tumor formation or other notable
371 proliferative changes. Injected hESC-RPE cells survived for the full 26 weeks in some
372 animals. Transplantation of undifferentiated hESCs under the same conditions was
373 associated with teratoma formation as well as unclassified mesenchymal tumors, peri-
374 lenticular mesenchymal hyperplasia, and an increase in the incidence and severity of
375 degenerative changes in the treated eye (fig 3C and 3D).

376

377 Owing to tumor formation by undifferentiated hESCs, the major safety concern
378 became the potential for survival and persistence of undifferentiated hESCs through the
379 manufacturing process. In-process testing for undifferentiated cells is essential in the
380 manufacture of any cell product from pluripotent cells for human transplantation. Spiking
381 studies and single-cell labeling studies demonstrated no detectable undifferentiated cells in
382 the final product. Even when we contaminated primary foci of hESC-RPE cells with up to
383 50% undifferentiated hESCs, no pluripotent hESC cells were present by the end of the
384 expansion phase. Furthermore, hESCs were not viable when dissociated and seeded into
385 RPE expansion conditions. Thus, we demonstrated that undifferentiated hESCs were not
386 detectable at stage 4 of the production process, and that the RPE differentiation medium
387 does not support the survival of hESCs.

388

389 Our preclinical studies in pigs investigated surgical feasibility, biodistribution, and
390 toxicity. We showed consistent facilitated mechanical delivery of the RPE patch in all 20
391 pigs operated on in the GLP final study using our purpose-built tool. Implantation of the
392 control membrane without cells led predictably to a foreign body reaction, but this was
393 minimal when RPE cells covered the membrane. The presence of RPE was also
394 associated with persistence of the native photoreceptor layer and less retinal atrophy than
395 membrane alone in both pig and clinical studies (Figures 3H, 3I, 4c1, 4c2, 4d1, 5c1, 5c2
396 and 5d1).

397

398 A qPCR analysis of systemic biodistribution in pigs at 26 weeks after implantation of
399 one clinical-sized graft (~100,000 hESC-RPE cells) showed no evidence that cells
400 migrated or survived away from the site of administration. The lack of distribution of the

401 cells is consistent with previous studies on ocular administration of RPE cells ¹⁹. This is
402 also supported by our NIH III mouse study, in which teratomas from undifferentiated
403 hESCs were found only in the eye, where the cells had been administered, and not in
404 tissues distal to the site, suggesting that undifferentiated hESCs do not migrate or do not
405 survive away from the site of implantation.

406

407 In the GLP pig study with no immunosuppression, no definitive hESC-RPE cells
408 were identified at 26 weeks, whereas persistent hESC-RPE cells were found at 6 weeks in
409 the earlier pig studies in which some animals were immunosuppressed perioperatively),
410 and at 26 weeks in the NIH III mouse teratoma studies (immune-deficient animals).
411 Histology of the implanted patch at 6 weeks from animals in the first pig study showed
412 persistence of human RPE and support of normal retinal architecture relative to animals
413 receiving the membrane alone (Fig 7H and I.). Microscopic findings consistent with a
414 localized chronic inflammatory reaction around the polyester membrane were present in
415 animals from both groups in the GLP pig study at 6 months, in the absence of any RPE cell
416 cover. There was no difference in the incidence or severity of the inflammatory reaction in
417 either group.

418

419 In the human clinical trial, the transplanted RPE patch survived, as demonstrated by
420 a clear RPE signal on OCT for 12 months and the visible persistence of a pigmented
421 cellular monolayer, although some of these cells may have been pigmented macrophages.
422 There was evidence of early auto-fluorescence in both patients 1 and 2, suggesting that
423 RPE phagocytosis has commenced ^{20, 21}. The retina over the patch was thinned in patient 1
424 but not in patient 2, which may reflect a difference in pre-operative disease or level of
425 microtrauma from manipulation during surgery. It is possible that the decrease in central
426 pigmentation in patient 1 represented cell loss from delayed rejection. Both patients
427 retained features of normal architecture and visible areas of the ellipsoid zone.
428 Furthermore, there was clear evidence of retinal function over the patch, as demonstrated
429 by fixation microperimetry, which showed focal sensitivity, and increased visual acuity and
430 reading speed, all of which were sustained or improved over the 12 months of follow-up.
431 However, in the absence of a control, proving the improvement was due to the transplant is
432 not possible. The improvement in reading exceeded that of similar cases in the submacular
433 surgery trial ²². Visual function remained variable across the transplanted area, with poor

434 visual function and thinning centrally in patient 1, which we feel may reflect intra-operative
435 surgical trauma.

436

437 While histological evidence of hESC-RPE survival is not possible, the extensive
438 number of structural and functional features discussed above support the conclusion that
439 the hESC-RPE survived. Furthermore, the presence of transplanted hESC-RPE cells
440 immediately adjacent to the neuroretina suggest that they are associated with retinal
441 function over the patch. We did not intentionally remove the subretinal choroidal neo-
442 vascular membrane, but cannot exclude that it was removed inadvertently. Therefore, the
443 effect of the original choroidal neovascular membrane on function remains ambiguous.
444 However, the co-localization of choroidal perfusion, survival of hESC-RPE, retinal
445 sensitivity, and presence of photoreceptors strongly support the conclusion that the visual
446 improvement and stability was associated with the transplanted RPE patch.

447

448 Retinal detachment was the most severe clinical complication seen. The GLP pig
449 studies had two retinal detachments in 20 animals, which is similar to the rates reported for
450 early human autologous transplantation and translocation surgeries^{33, 34}. The retinal
451 detachment in patient 2 of the clinical trial was likely a PVR-associated detachment that
452 occurred between 4 and 8 weeks. It was treated with a single operation using standard
453 techniques. The retinal detachment did not extend far enough to reach the patch.
454 However, despite the delivery of RPE cells on a patch and in a protected delivery device,
455 both of which minimize the shedding of RPE cells into the vitreous, we cannot be certain
456 that no hESC-RPE cells were released nor that they did not contribute to the risk of
457 developing PVR. The true surgical risk can be assessed accurately only with a larger
458 series of patients.

459

460

461 In patient 1, there was an area of native RPE over the patch that was clearly
462 delineated and separate from the large area over the patch where the native RPE was lost
463 and only hESC-RPE was present (Figs 3 and 5). Notably, the patient fixated not over
464 native RPE but over the RPE patch. Subjectively, patient 1 acknowledged that vision is
465 improved relative to the pre-operative state and that she can see letters and read directly
466 with the central vision; however, she described troublesome distortion, similar to that
467 experienced by patients who have had previous retinal detachment or wet AMD. The

468 second patient described his vision as continuously improving but also being 'dimmer' than
469 before the onset of the disease, which is consistent with his longer-standing disease at
470 presentation and, likely, more damaged neuroretina.

471

472 Reporting these first two cases at 12 months is valuable because the functional
473 improvement, robust imaging data, lack of major safety concerns, and demonstration of
474 sufficiency of local immunosuppression represent steps in support of hESC-based
475 regenerative therapy for AMD and other diseases of the eye. We show that differentiation
476 of hESCs into a therapeutic cell with delivery, survival and rescue of vision in very severe
477 disease is feasible. Although 12 months is sufficient to begin to describe cell survival and
478 clinical outcomes, it is early in terms of safety monitoring, especially for late teratoma
479 formation. The patients will be followed for a total of 5 years after surgery. These two early
480 cases are also instructive as they show an encouraging outcome despite very advanced
481 disease, which increases the complexity of surgery and involves more-damaged
482 neuroretina. Additionally, there was no evidence of recurrence of the neovascular
483 membrane and no need to administer angiogenesis inhibitors to either patient at 12
484 months, although with only two cases it is difficult to attribute this to the transplant.

485

486 The role of immune privilege in the subretinal space remains ambiguous, and the
487 immunological effect of the surgery and patch transplant is unknown. Stability of the
488 transplant was achieved with immunosuppression consisting of perioperative oral
489 prednisolone and long-term intraocular steroid implants. For patient 2, who has type II
490 diabetes, there was a period of poor blood sugar control with the need to add insulin, due
491 to the systemic steroid use. Given the concerns about long-term systemic
492 immunosuppression, a notable finding of this study is that the transplanted hESC-RPE
493 cells survived at least 12 months with only local immunosuppression. Although long-term,
494 local immunosuppression can be provided in the eye without systemic side effects, the
495 possibility of long-term ocular morbidity remains. In our two patients, there was no
496 associated intraocular pressure rise or need for pressure-reducing medication.

497

498 Stem-cell-based tissue transplantation is a potentially effective treatment strategy for
499 neurodegenerative or other diseases with irreversible cell loss. Here we addressed
500 challenges related to engineering, manufacturing and delivering a clinical-grade hESC-
501 RPE patch, leading to stabilization and improvement of vision for at least 12 months in two

502 subjects with severe vision loss from AMD. These findings support further investigation of
503 our approach as an alternative treatment strategy for AMD.

504

505 **Competing interests**

506 J.K., M.B., M.F., J.S., T.H., G.F., M.W., P.T.L., and P.W. were all employees of Pfizer
507 during the period of this clinical trial. This study was sponsored by Pfizer Inc.

508

509 L. d C and P.J.C. are named on 2 patents lodged by University College London (UCL)
510 Business. They are Patent Application No. PCT/GB2009/000917 (for the patch) and
511 International Patent Application No. PCT/GB2011/051262 (for the surgical tool).

512

513 **Author Contribution**

514 L. d C*,‡, P.T.L., P.W., and P.J.C. designed all of the animal studies and clinical study,
515 developed the methodology for these studies, completed pig surgery*, completed human
516 surgery‡, collected the data, performed the analysis, and wrote the manuscript.

517

518 K.F., J.K., A. A., ** A.Ve., J.T.D., B.N., S.M.H., S.B.G., A-J.F.C., A.Vu. **, C.M. R., M.B.,
519 M.F., J.S., T.H., A.W. Developed, isolated and prepared the hESC – RPE and completed
520 the engineering of the hESC-RPE patch; assisted design and assisted in completing the
521 mouse and pig studies, completed mouse surgery**; collected the data, performed the
522 analysis, and assisted in writing of the manuscript.

523

524 O.G., Y.H.L., A.A., A.T., G.F., M.W., A.G.R., G.E. H., M.S.S., Assisted in design of the
525 clinical study, developed the methodology, collected the data, performed the analysis, and
526 assisted in writing the manuscript.

527

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672

673 **Legends**

674

675 **Figure 1** Generation of hESC-derived RPE for the manufacture of an advanced therapeutic
676 medicinal product to treat AMD.

677 This figure outlines the chronological process for manufacturing the RPE patch from SHEF-
678 1.3 hESCs. The first four rows show the stages of differentiation; the coating on the plastic-
679 ware for each step; the media used at each step; and the tests and checks performed at
680 each step. The bottom row shows brightfield images illustrating each stage: **[AU: add**
681 **column to image and label each row]** (i) hESC colonies expanded on recombinant
682 human vitronectin (VTN-N). (ii) Spontaneously differentiated RPE cells appear as distinct
683 pigmented foci. **[AU: line is offset; does that mean E8 medium is used at the beginning**
684 **of SHEF-1.3 differentiation? Please clarify]** (iii) These foci are manually dissected,
685 dissociated, and filtered to achieve a pure single-cell type RPE population, which is seeded
686 onto plates, where expanding RPE cells establish their classic pigmented, cobblestone
687 morphology. (iv) Fully differentiated RPE seeded at confluence. **[AU: add PET to text in**
688 **figure as in v; deleted here]** (v) A therapeutic 'patch' consisting of the RPE monolayer
689 immobilized on the vitronectin-coated PET membrane that has been cut from the transwell.
690 (vi) Following release tests the advanced therapeutic medicinal product is supplied to the
691 surgical team in a custom-manufactured single-use sterile container in saline, where it is
692 viable for up to 8 h. PMEL17, premelanosome marker 17; ICC, immunocytochemistry; ISH,
693 *in situ* hybridization; PET, polyethylene terephthalate; VIR test, visual inspection release
694 test

695

696 **Figure 2** Characterization of human hESC-derived RPE.
697

698 (a) Confocal bright-field immunofluorescent micrographs depicting staining for typical
699 regional RPE markers as part of the characterization of the monolayer as RPE (each
700 represents a single experiment with no repeats). Scale bars, 25 μ m. Predominantly apical
701 PEDF and basal BEST1 expression confirms polarity of these cells. PMEL17 expression
702 confirms presence of premelanosomes, and ZO1 expression allows easy identification of
703 tight junctions. Absence of Ki67 confirms that cells are not proliferating. Other characteristic
704 RPE markers (CRALBP, MITF, OTX2), crucial for melanogenesis and visual cycle, are also
705 present. Immunocytochemistry was performed once on SHEF-1.3-derived RPE cells.
706 BEST1, bestrophin 1; CRALBP, cellular retinylaldehyde-binding protein; DAPI, diamidino-2-
707 phenylidole; MITF, microphthalmia transcription factor; OTX2, orthodenticle homeobox 2;
708 PEDF, pigment epithelium-derived factor; PMEL17, premelanosome protein 17; ZO1,
709 zonula occludens 1.

710

711 (b) Quantification of PEDF secretion in spent culture medium during late expansion phase,
712 analyzed using an ELISA assay. The classical RPE PEDF secretion asymptote is visible
713 from around 3 weeks onwards. Each different colored box represents a separate batch of
714 tested cells.

715

716 (c) Electron micrographs of RPE cells, illustrating the classical ultrastructure associated
717 with normal RPE function including tight junctions (white arrows; i and iii), basal infoldings
718 (black arrows; i and iv), apical microvilli (i–iii), and melanin granules (i–iii) revealing various
719 stages of melanogenesis (ii; stage II/III, III melanosomes and stage IV mature
720 melanosomes). Transmission electron microscopy was performed once on SHEF-1.3-
721 derived RPE cells. N, nucleus; AmV, apical microvilli. Images represent a single
722 experiment with no repeats.

723

724 (d) SHEF-1.3-derived RPE cells internalize photoreceptor outer segments (POS),
725 demonstrating SHEF-1.3-derived RPE phagocytosis. The extent of phagocytosis was also
726 examined after treatment of the SHEF-1.3 RPE with MERTK antibody (SHEF-1.3 RPE +
727 blocker). Data shown are mean internalized POS \pm s.e.m. ($n = 3$ biologically independent
728 cell cultures in each group). Pre-incubation with MERTK antibody had a significant effect
729 on the number of POS ingested by SHEF-1.3 RPE ($P = 0.0000103075196107$, two-tailed

730 Student's *t*-test, This was a single experiment with no replication. Below the graph,
731 confocal micrographs show the internalized POS with a green fluorescent marker (POS) in
732 SHEF-1.3-derived RPE and its relative absence in SHEF-1.3 RPE with MERTK antibody
733 (SHEF-1.3 RPE + blocker).

734 .

735 .

736 .

737 **Figure 3** Preclinical mouse teratoma and pig transplantation studies.
738
739 NIH III immune-deficient mice preclinical teratoma studies.
740 (a) Normal non-injected eye of a mouse (H&E).
741 (b) 6-week-old animal injected with hESC-RPE (no teratomas or proliferation were
742 observed in any animals from this group). Section taken 26 weeks after injection (H&E).
743 (c) An example of teratoma formation in an animal injected with undifferentiated hESC.
744 Section taken following euthanization of the animal prior to 26 weeks (H&E).
745 (d) An example of a mesenchymal tumor NOS in an animal injected with undifferentiated
746 hESC. Section taken at termination of the animal prior to 26 weeks (H&E).
747 (e, i) 6-week-old animal injected with hESC-RPE showing persistence of layers of
748 pigmented cells at week 26. (e, ii) 6-week-old animal injected with hESC-RPE and
749 examined with anti-human mitochondria IHC. Immunoreactivity for human mitochondria
750 seen as a brown chromophore (black arrows), demonstrating that the cells represent
751 implanted-hESC-derived RPE. (e, iii) 6-week-old animal injected with hESC-RPE and
752 examined with an isotype control relative to section in e, ii with no positive staining (black
753 arrows) using the same chromophore as in e, ii.
754 (f,g) *In vivo*, color fundus photographs of pig eyes from the GLP pig study taken 6 weeks
755 after transplantation surgery. (f) The picture shows an eye that has been implanted with a
756 'patch' consisting of an hESC-derived RPE monolayer on a human-vitronectin coated
757 polyester membrane. The patch is seen as a consistent and evenly pigmented cellular
758 covering over the entire area. (g) Uncoated patch with no cells, which has been implanted
759 as a control.
760 (h) Cresyl violet stain of pig retina at 6 weeks post-transplantation with a patch of RPE
761 implanted into the subretinal space. INL, inner nuclear layer; ONL, outer nuclear layer.
762 (i) Cresyl violet stain of pig retina at 6 weeks post-transplantation with a patch without cells
763 implanted into the subretinal space. GCL, ganglion cell layer; INL, inner nuclear layer. Note
764 the absence of the ONL.
765 (j) Unstained pig retina 6 weeks post-transplantation with a patch of RPE cells which are
766 pigmented (arrows highlight the position of the polyester membrane).
767 (k) Serial section from the same eye stained with anti-human TRA-1-85 indicates that the
768 cells are human in origin (arrows highlight the position of the polyester membrane).
769
770

771 **Figure 4.** Case 1: Pre- and post-operative imaging of the cell patch with structural and
772 functional outcomes at 12 months following surgery.

773
774 (a, i) Pre-operative color fundus photograph demonstrating the extensive sub-RPE and
775 submacular hemorrhage extending under the fovea secondary to wet AMD. (a, ii) Spectral
776 domain OCT (Spectralis, Heidelberg) showing pre-operative section with inset showing the
777 position of the slice. (b, i) 12 months' postoperative color fundus photograph showing the
778 patch covered with pigmented cells throughout. The test also shows microperimetry results
779 with two areas of demonstrated sensitivity (Nidek microperimetry) labeled x and y. The
780 circles represent the extent of the visual stimulus demonstrating that it falls completely
781 within the transplanted area. Inset § shows a magnified detail of the patch area with the
782 levels of sensitivity shown by microperimetry in decibels. Inset * shows a close up of the
783 fixation with the Nidek microperimeter fixation outcome superimposed. (b, ii) Spectral
784 domain OCT (Spectralis, Heidelberg) showing post-operative section with inset showing
785 the position of the slice. The patch is shown in position with thinned retina over the area of
786 the patch but an intact ellipsoid layer. (c, i,ii) Spectral domain OCT sections through x and
787 y, respectively. The area on the section corresponding to x and y is indicated with a bar.
788 The long red arrow in c, i and c, ii indicates the ellipsoid layer. The thin and bold green
789 arrows in c, i and c, ii indicate the synthetic membrane and the hESC-RPE, respectively.
790 (d, i) Images from the Rtx1 AO fundus camera. (Inset) Color fundus photo with
791 superimposed area of AO imaging. The dotted yellow circle corresponds to the area of
792 positive microperimetry sensitivity marked x throughout this figure. (d, i) Magnified image of
793 the area indicated by the dotted yellow line in g, i showing bright dots corresponding to
794 cone photoreceptors based on their size, inter-photoreceptor distance and organization
795 (blue arrows). (d, ii) AO image taken from a normal patient at the same point of the retina
796 as in g, ii for comparison. (e, i) Early-phase fundus fluorescein angiogram (FFA) showing
797 early choroidal perfusion in the area of the patch and specifically the two points of
798 demonstrated sensitivity x and y. The dark areas over the transplanted patch, at the nasal
799 end of the patch and the superior border, represent areas of native RPE over and under
800 the patch, masking emission rather than absent choroidal perfusion. (e, ii) Late-phase FFA
801 confirming choroidal perfusion and no leakage under the patch. (e, iii) SLO
802 autofluorescence over the patch, including in areas x and y, showing dim but visible
803 autofluorescence.

804
805 **Figure 5** Case 2: Pre- and post-operative imaging of the cell patch with structural and
806 functional outcomes at 12 months after surgery.

807
808 (a, i) Preoperative color fundus photograph of the extensive sub-RPE and submacular
809 hemorrhage extending under the fovea, secondary to wet AMD. (a, ii) Spectral domain
810 OCT (Spectralis, Heidelberg) showing pre-operative section with inset showing the position
811 of the slice. (b, i) 12 months post-operative color fundus photograph showing the patch
812 covered with pigmented cells throughout. There is extensive epiretinal fibrosis associated
813 with the extent of the disease and a retinal detachment that had been successfully
814 reattached. The test also shows microperimetry (Nidek microperimetry) results with two
815 areas of demonstrated sensitivity labeled x and y. Inset § shows a magnified detail of the
816 patch area with the levels of sensitivity shown by microperimetry in decibels. Inset * shows
817 a close-up of the fixation with the Nidek microperimeter fixation outcome superimposed.
818 The circles represent the extent of the visual stimulus demonstrating that it falls completely
819 within the transplant area. (b, ii) Spectral domain OCT (Spectralis, Heidelberg) showing
820 post-operative section with inset showing the position of the slice. (c, i,ii) Spectral Domain
821 OCT cuts through area x and y, respectively, as indicated. The detail shows the Spectral
822 Domain OCT sections through x and y, respectively. The area on the section
823 corresponding to x and y is indicated with a bar representing the area. The long red arrows
824 indicate the ellipsoid layer. The thin and bold green arrows indicate the synthetic
825 membrane and the hESC-RPE respectively. (d, i) Images from the Rtx AO fundus camera.
826 Inset - color fundus photo with superimposed area of AO imaging. The magnified image of
827 the area indicated shows bright dots corresponding to cone photoreceptors based on their
828 size, inter-photoreceptor distance and organization. (d, ii) An Rtx AO fundus camera image
829 taken from a normal patient at the same point of the retina as in d, i for comparison. (e, i)
830 An early phase Fundus fluorescein angiogram (FFA) showing early choroidal perfusion in
831 the area of the patch and particularly the two points of demonstrated sensitivity x and y.
832 The dark areas temporal and superior to the transplanted patch likely represent areas of
833 choriocapillaris loss associated with the primary pathology and surgery. (e, ii) Late phase
834 FFA confirming choroidal perfusion and no leakage under the patch. (e, iii) SLO
835 autofluorescence image over the patch including areas x and y demonstrating
836 autofluorescence over parts of the patch.

837

838 **Figure 6.**

839

840 a. A sequence of colour photographs of the transplanted patch in case 1 at 4, 24, and 52

841 weeks. The sequence shows the centrifugal expansion of the pigmented areas around the

842 patch and the stability of pigmented areas on the patch. It also shows the regression of the

843 host RPE on the patch, with the original margin indicated by the dotted line. b. A sequence

844 of colour photographs of the transplanted patch in case 2 at 4, 24, and 52 weeks. The

845 sequence shows the centrifugal expansion of the pigmented areas around the patch and

846 relatively stable areas of pigmentation on the patch. The expansion of pigmented areas in

847 both 6a and b stabilized by week 24.

848

849

850

851 **Figure 7.**

852

853 a. Best Corrected Visual Acuity (BCVA) over 12 months for patient 1. b Reading speed

854 over 12 months for patient 1. c. BCVA over 12 months for patient 2. d. Reading speed over

855 12 months for patient 2.

856

857

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859

860

861 **Online Methods.**

862

863 **Ethics statement**

864

865 All animal (mouse and pig) procedures were conducted in accordance with the provisions
866 of the United Kingdom Animals Scientific Procedures Act (ASPA) 1986. The animal
867 experimentation under the ASPA is overseen by the Home Office, UK government, in
868 England.

869

870 The mouse teratoma studies were carried out by the Independent Contract Research
871 (CRO) Organization Huntington Life Sciences (HLS), now Envigo (Alconbury/Huntingdon,
872 Cambridgeshire, UK). (HLS Animal Project - Licence number PCD 70/8702).

873

874 The pig surgery was carried out under GLP conditions at the Northwick Park animal
875 experimental surgery facility (Northwick Park and St Mark's Hospital, Harrow, Middlesex,
876 UK). Pigs were cared for in an independent GLP registered facility including throughout the
877 6-month's follow-up period. The animal reporting including organ sampling was carried out
878 independently by HLS.

879

880 **Clinical trial**

881 Pfizer Inc. sponsored and was the sole funding source of the clinical trial. Two subjects out
882 of a planned 10 were enrolled before Pfizer suspended funding for strategic commercial
883 reasons unrelated to this trial. The cells for transplantation were prepared to the state of
884 confluent RPE at Roslin Cells Ltd (Edinburgh, United Kingdom,) before transport to
885 London. The preparation and cutting of the patch was completed at Cells for Sight (Institute
886 of Ophthalmology, Bath St, London, UK).

887

888 Approval was granted from the U.K. Medicines and Health Products Regulatory Authority
889 (MHRA), the Gene Therapy Advisory Committee (GTAC), the Moorfields Research
890 Governance Committee and the London - West London & GTAC Research Ethics
891 Committee. The study complied with Good Clinical Practice guidelines according to the
892 European Clinical Trials Directive (Directive 2001 EU/20/EC), the Declaration of Helsinki
893 and has an independent External Data Monitoring Committee (E-DMC). The data
894 monitoring committee had three representatives who were retinal surgeons with two having

895 specialty expertise in ocular oncology and the third in ocular immunology. Informed
896 consent was obtained from each patient. The study compliance with protocol was reviewed
897 regularly, and none of the recorded protocol deviations had an impact on subject safety or
898 study integrity

899

900 **Reporting Summary**

901 The *Nature Biotechnology* Reporting Summary has been completed and submitted with
902 this article

903

904 **Statistics**

905 The only statistical analysis reported was for the phagocytosis study (Figure 2d). Data
906 shown are mean \pm s.e.m., $n = 3$ biologically independent cell cultures in each group. As the
907 n is less than 10, individual data points are indicated. Analysis was by two-tailed Student's
908 *t*-test with $P = 0.0000103075196107$. This was a single experiment with no replication.

909

910 **Preparation of hESC-RPE (GMP conditions and facility).**

911 The original cell source, the SHEF1 hESC (NIBSC - UK Stem Cell Bank -
912 <http://www.nibsc.org/ukstemcellbank>) line was expanded according to GMP guidelines at
913 the Stem Cell Derivation Facility, Centre for Stem Cell Biology (CSCB), University of
914 Sheffield³⁵. The SHEF 1 and 1.3 cell lines were authenticated by A Short-Term Repeat
915 Analysis fingerprint, which was performed by University of Wisconsin Hospital and Clinics.
916 The analysis showed an identical STR genotype profile across 8 human STR loci for both
917 SHEF-1 and SHEF-1.3. The SHEF-1.3 cell line was derived from the expanded SHEF1
918 hESC cells under the same GMP conditions at the same facility. The passage and
919 expansion of SHEF-1.3 hESCs was carried out in recombinant human vitronectin (VTN-N)
920 (Life technologies)-coated culture vessels. Medium was removed from cultures and hESCs
921 were washed with PBS-/-, incubated with EDTA (Sigma) until the cell colonies begin to
922 detach. Cells were resuspended in Essential 8 (E8) medium (Life Technologies) and
923 seeded onto VTN-N-coated culture vessels. hESC were replenished with E8 medium daily,
924 and passaged every 3–4 d depending on visual observations of colony morphology and
925 size.

926

927 hESC differentiation to RPE was carried out in flasks coated with plasma-derived
928 vitronectin (Amsbio). Sets underwent regular media replenishment with E8 medium for up

929 to 14 d until hESC colonies were approximately 80 to 100% confluent and then transitioned
930 to TLP medium consisting of 389ml of Knockout DMEM, 1 ml of 2-Mercaptoethanol, 5ml of
931 NEAA, 100 ml of Knockout serum and 5 ml of L-glutamine per 500 mL.
932 (Invitrogen – LifeTechnologies (Thermo Fisher Scientific). Cells were maintained on a twice-
933 weekly media replenishment regimen. RPE cells appear in culture as distinct pigmented
934 foci, visible to the naked eye, which continue to expand in diameter and can be maintained
935 in this culture system for up to 22 weeks.

936

937 RPE foci were manually isolated using sterile microblades (Interfocus) and collected in TLP
938 medium. Pooled foci were washed with PBS-/- and incubated at 37 ± 2 °C with Accutase
939 (Sigma) until a cell suspension is observed. The suspension was then passed through a
940 70µm cell strainer (Corning), washed in TLP medium and counted. The cells were plated
941 on CELLstart- coated (Life Technologies) 48-well plates at 4.8×10^4 cells/well. Cells were
942 maintained on a twice-weekly media replenishment regimen of TLP medium until they
943 formed a confluent, pigmented cell sheet with cobblestone morphology, generally for a
944 minimum of 5 weeks.

945

946 To ensure safety and purity of the RPE, it was essential to exclude the possibility of
947 undifferentiated hESCs being administered to a patient. In situ hybridization was used to
948 assess hESC impurity. Samples of differentiated cells were prepared as a monolayer on
949 microscope slides. Specific oligo probes against LIN28A mRNA were used to evaluate the
950 presence of hESC in the RPE population. LIN28A stained cells were identified using a
951 nuclear stain. If any LIN28A positive cells were detected, the RPE batch would be rejected.
952 Differentiated RPE were further assessed using immunocytochemistry against the
953 melanosome-specific protein PMEL17 (Dako), an RPE cellular marker. PMEL17 positively
954 stained cells were counted and expressed as a percentage of total cells. Upon testing of
955 cells on representative cell patches, the RPE purity ranged from 99.8 to 100% on positive
956 staining of PMEL17 and no LIN28A positive cells were detected.

957

958 RPE cells were assessed using a light microscope for pigmentation, cobblestone
959 morphology, health and signs of contamination and processed further only if they passed
960 this visual check. Media was removed, cells were washed with PBS-/-, and incubated at
961 37 ± 2 °C with Accutase for 1–2 h until a cell suspension was observed. The suspension
962 was passed through a 70µm cell strainer, washed in TLP medium and then counted. Cells

963 were subsequently seeded (1.16×10^5 cells/well) into custom manufactured transwells, with
964 polyester membranes coated in plasma-derived human vitronectin. The membrane was
965 10 μ m thick polyethylene terephthalate (PET), with a 0.4 μ m pore size at a density of 1×10^8
966 pores/cm² (Stereitech, Kent, Washington, USA). TLP medium was added to the outer well
967 containing the insert, and the plate then incubated at 37 \pm 2°C. The RPE cells were
968 maintained with twice weekly TLP media replenishment until required for drug product
969 manufacture.

970

971 On the day of surgery, the transwell was removed from the culture plate and placed directly
972 onto the cutting device. The patch is cut from the membrane and is then placed into the
973 storage solution (0.9% sodium chloride) within the storage container that is then sealed.
974 (Figure 1a.) The patch is assessed visually through the clear lid of the storage container for
975 integrity, pigmented cell coverage and viability. The patch was transported out of the GMP
976 facility and transferred to the operating theatre.

977

978 **Patch cell count.**

979 The theoretical cell count on each patch was calculated using an observed cell diameter of
980 14 μ m and this gives approximately 100,000 cells per patch. To complement this, two
981 patches from preclinical production batches had the cells dissociated and counted and
982 gave counts of 110,000 and 120,000 cells total (i.e. a cell diameter of approximately 12-
983 13 μ m). The theoretical cell count of 100,000 was within the confidence intervals from
984 these two counts, and so was selected.

985

986 **Immunocytochemistry.**

987 Immunostaining was performed as described previously¹⁶. Briefly, cells were fixed in cold
988 4% paraformaldehyde (PFA) in 0.1M phosphate buffer and then blocked and incubated
989 with an appropriate combination of the following primary antibodies: TRA-1-60 (Life
990 Technologies); Ki67 (VectorLabs); CRALBP (Thermo Scientific); PMEL17 (Dako); MERTK
991 (Abcam); ZO-1 (Life Technologies); OTX2 (Millipore); Bestrophin (Millipore) and PEDF
992 (Millipore). They were then incubated in the appropriate secondary antibodies including
993 AlexaFluor 647 conjugated donkey anti-mouse Ig-G (Life Technologies), 594 conjugated
994 donkey anti-rabbit Ig-G (Life Technologies) and Dylight 488 conjugated donkey anti-mouse
995 Ig-M (Startech). Nuclei were stained using Hoechst 33342 (Life Technologies).

996

997 **Transmission electron microscopy (TEM)** RPE cells immobilized on a membrane were
998 fixed in Karnovsky's fixative and prepared for TEM as described previously¹⁶.
999

1000 **PEDF assay** on spent media was completed using a developmental ELISA (Meso Scale
1001 Discovery Platform S600, Mesoscale, USA) according to manufacturer's instructions.
1002

1003 **Phagocytosis assay method**

1004 This was based on a previously described method³⁶. Purification of photoreceptor outer
1005 segments (POS) was performed as described previously³⁷. The POS were labeled as
1006 described previously³⁸. The labeled POS were seeded onto hESC-RPE monolayer patches
1007 grown on plasma-derived human vitronectin at 1 x 10⁷ POS/ml. In one condition the cells
1008 were pre-incubated with 1:50 anti MERTK antibody (ab52968, Abcam) to block
1009 phagocytosis for one hour. The cells were incubated at 37°C in 5% CO₂ for 6 hours. The
1010 samples were fixed and stained as described previously³⁸. To quantify phagocytosis, 3
1011 distinct areas per patch of hESC-RPE or hESC-RPE with blocker (MERTK antibody) (n=3)
1012 were imaged blind on the Zeiss 710 confocal. The total number of internalized fluorescent
1013 POS were counted in a 135µm x 135µm area, using the orthogonal view function.
1014

1015 **Preclinical animal safety studies**

1016

1017 **Mouse teratoma studies.**

1018 The cells tested were undifferentiated SHEF-1.3 pluripotent hESCs (hereafter 'hESCs'),
1019 and SHEF-1.3 hESC-derived RPE ('hESC-RPE'). The cells were grown at a Pfizer facility
1020 before transfer to HLS. They were injected into the eyes of NIH III Immune-Deficient Mice
1021 (strain Crl:NIH-Lyst^{bg}Foxn1^{nu}Btk^{xid}, 6-7 weeks old; Charles River, UK) at HLS. The
1022 injections were carried out by Antony Vugler & Ahmed Ahmado from University College
1023 London (UCL). Histological and pathological analyses were carried out by HLS, who
1024 provided a report on the study.
1025
1026

1027 The second study was completed as there were fewer tumors in female animals in the first
1028 study. It was concluded that this was most likely linked to a decline in cell suspension
1029 quality for dosing of the females, rather than an actual sex difference. The second study,
1030 focused on female animals. In addition, we studied the relative rate of teratoma formation

1031 following subretinal, subcutaneous (SC) or IM injection of undifferentiated hESCs in female
1032 NIH-III mice. In the second study five males were also dosed with undifferentiated hESCs
1033 subretinally.

1034
1035 In the third study, under GLP conditions, NIH III mice were administered suspensions of
1036 hESC-RPE cells or suspensions of undifferentiated hESCs as a positive tumorigenic
1037 control, with all injections being delivered subretinal. Other controls carried out in all the
1038 mouse teratoma studies are listed in the tables below. Animals were followed for up to 26
1039 weeks, allowing for the assessment of tumors associated with the administration of the test
1040 cells, prior to the animals having an excessive burden of spontaneous tumors. The
1041 numbers of animals injected, the site and sex are listed in the following table.

1042

1043

1044 **Mouse teratoma studies**

1045

1046 **First study: 2-Month Pilot Study of the Teratoma Formation Potential of hESCs in NIH III
1047 Immune-Deficient Mice**

1048

Test condition	*Injected Number / sex animals
1. Untreated Control	5/sex (10 total)
2. DMEM Control – Subretinal	5/sex (10 total)
3. hESCs at 45,000 M/44,000 F cells – Subretinal	15/sex (30 total)
4. hESCs at 88,000 M/68,000 F cells – Subretinal	15/sex (30 total)
5. Vehicle – IM	5/sex (10 total)
6. hESCs at 88,000 M / 68,000 F cells – IM	5/sex (10 total)

1056

1057

1058 **Second Study: 2-Month Pilot Study of the Teratoma Formation Potential of SHEF1-Cells in
1059 Female NIH III Immune-Deficient Mice**

1060

Test condition	*Injected Number / sex animals
1. DMEM – Subretinal	5F (5 total)
2. hESCs at 35,600 cells – Subretinal	5/M (20 total)
	15/F
3. Matrigel – IM	5 F (5 total)
4. HESCS at 35,500 cells – IM	10F (10 total)
5. hESCs at 822,500 cells – IM	10F (10 total)
6. Matrigel – SC	5F (5 total)
7. hESCs at 35,500 cells – SC	8F (8 total)
8. hESCs at 822,500 cells – SC	12F (12 total)

1071

1072

1073 **Third Study (GLP conditions) Teratoma Formation Potential of hESC-RPE in NIH III Immune
1074 Deficient Mice for 26 Weeks Following Intraocular Dosing**

		Injected Number / sex animals	
1075	Test condition		
1076	1. KO DMEM Control	10/sex	(20 total)
1077	2. mTeSR™ Control	10/sex	(20 Total)
1078	3. hESC-RPE at 5340 cells* in KO DMEM	20/sex	(40 Total)
1079	4. hESC-RPE at 60,400 cells* in KO DMEM	20/sex	(40 Total)
1080	5. hESCs at 4510 cells§ in mTeSR	19 M/20 F	(29 Total)
1081	6. hESCs at 42,800 cells§ in mTeSR	20 M/19 F	(29 Total)

1082 DMEM = Dulbecco's Modified Eagle Medium; KO DMEM = Knockout DMEM;

1083 IM = Intramuscular; SC = Subcutaneous. M = Male; F = Female;

1084 RPE = Retinal Pigment Epithelium;

1085 § Represents the average number of cells administered to the group.

1086

1087 **Necropsy and histology.**

1088 At the end of the study animals were subject to a detailed necropsy and macroscopic
 1089 examinations were performed on all treated and control animals. The following tissues
 1090 were processed for histological examination: abnormal masses, epididymides, eyes, heart,
 1091 kidneys, liver, lymph nodes (mesenteric, submandibular), optic nerves, ovaries, spleen,
 1092 sternum with bone marrow, testes. Tissues and organs were collected and fixed in 10%
 1093 neutral buffer except the eyes and optic nerves which were fixed in Davidson's fluid.
 1094 Following histological processing and review any teratomas or abnormal masses were
 1095 subject to immunohistochemistry (IHC) (anti-human mitochondrial marker staining), using a
 1096 validated method to confirm whether these were of human or mouse origin.

1097

1098 **Pig transplantation feasibility studies.**

1099 The analysis of the biodistribution data in pig tissues (looking for the evidence of human
 1100 cells in different organs) was done using qPCR by the independent CRO Aros (Aarhus,
 1101 Denmark) with reports provided.

1102

1103 Surgical safety, successful delivery of the RPE patch, local and systemic biodistribution
 1104 and toxicology were examined in these studies. The pig was selected since the size of the
 1105 pig eye allowed for the administration of the intended full-size patch and would allow the
 1106 study of surgical feasibility, biocompatibility and biodistribution. All pig studies described
 1107 were performed by the same surgeon who operated in the two cases described in the initial
 1108 clinical trial. In the second pig study the same clinical surgical technique was used as in the
 1109 human trial.

1110

1111 **First pig study.**

1113 In this study, SHEF1-derived RPE cells were seeded onto a BD Matrigel (BD Biosciences,
1114 Erembodegem, Belgium)-coated polyester membrane and manually implanted as a 1x3
1115 mm membrane into the subretinal space of pig eyes via 20G pars plana vitrectomy, bleb
1116 detachment with retinotomy followed by air tamponade. Twenty animals underwent surgery
1117 and 12 of these received *hESC-derived-RPE* cells on the membrane, 2 at each time point,
1118 with the remainder receiving a membrane without cells, 1 at each time point except 6
1119 weeks where there were 3 animals. Six of the animals received immunosuppression with
1120 oral cyclosporine (650 mg for up to 2 weeks following surgery). Animals were observed,
1121 euthanized and enucleated at 2 hours, 2 d, 1, 2, 4, and 6 weeks. Eyes were examined by
1122 light and electron microscopy.

1123

1124 **Second pig study.**

1125 *Safety/Biodistribution Study of therapeutic patch in Pigs for 26 Weeks*

1126 *Following Surgical (Intraocular), subretinal Implantation*

1127

1128 1. Control (vitronectin coated polyester membrane only)	5/sex (Total 10)
1129 2. PF-05206388 1 graft (100,000 cells plus vitronectin	5/sex (Total 10)
1130 coated polyester membrane)	

1131

1132 **Toxicity.**

1133 The feasibility of the surgical procedure and the general safety of PF-05206388 was
1134 assessed in studies performed in the Large White/Landrace hybrid pigs (in all cases).

1135

1136 In the GLP pig study, a group of 5 male and 5 female pigs were administered a patch at a
1137 dose of 1 graft in the left eye (approximately 100,000 SHEF-1.3 derived RPE cells on a
1138 vitronectin coated polyester membrane) and observed for 26 weeks (6 months). A similarly
1139 constituted control group of 5 male and 5 female animals received the vitronectin coated
1140 polyester membrane only. The membrane with or without cells was placed into the
1141 subretinal space in all 20 pigs using the same tool and surgical technique as in the clinical
1142 trial. Animals received Prednisolone (1 mg/kg) orally 7 d before surgery, which continued
1143 for 14 d post operatively, intravitreal 4 mg Triamcinolone Acetonide (TCA) during the
1144 surgery, Sub tenon TCA 40mg at the end of surgery, and Carprofen (4 mg/kg) was given
1145 orally postoperative on day 2 of the study. Eye drops of 0.1% dexamethasone, 0.5%

1146 hypromellose and 0.35% neomycin were administered to all the pigs 4 times per day for 17
1147 d post-surgery.

1148

1149 **Systemic distribution of cells.**

1150 The distribution of the hESC-RPE cells was evaluated as part of the pivotal 26-week
1151 toxicity study in pigs. The biodistribution was evaluated using qPCR techniques and the
1152 human cell markers huGAPD, huPEDF, HSSRY, huPOU5F1, and huOTX1/2 and the pig
1153 marker, pigACTB. A total of 60 samples from each animal (5/sex/group) that included the
1154 following: adrenal, bone marrow (rib and femur), brain (7 sections), heart (5 sections),
1155 kidneys (5 sections), liver (6 sections), lungs (5 sections), lymph nodes (4 nodes, total 8
1156 sections), optic nerve (left and right, proximal and distal to eye), spleen (5 sections),
1157 thymus (3 sections) were evaluated. To avoid a high number of false positives, positives in
1158 at least 2 individual human markers in the same sample were required. There were no
1159 animals that received the matrix only that had an increase in amplifying human transcripts.

1160

1161 **Cell spiking studies.**

1162

1163 The effect of deliberately spiking hESCs into RPE was assessed. Different proportions (0,
1164 1, 10, 20, 50 or 100%) of hESCs were mixed with dissociated RPE foci (P0 cells), seeded
1165 into 96 well plates at 38,000 cells/cm² and cultured for 6 weeks (P1 cells) using standard
1166 RPE culture conditions. Feeder-free hESC were used for spiking to eliminate counting
1167 inaccuracies due to the presence of fibroblast feeder cells. Plates were fixed and stained
1168 for TRA-1-60 or PMEL17 and the corresponding no primary antibody controls (n=4 plates,
1169 1 well/plate stained with each antibody/control). A single cell suspension of hESCs was
1170 also seeded onto Matrigel in mTeSR1 media with ROCK inhibitor 2 d before fixing as a
1171 positive control for TRA-1-60 staining.

1172

1173 **Flow cytometry.**

1174 Cells were washed with PBS and treated with Accutase to produce a single cell
1175 suspension, before pelleting and resuspended in 3% BSA/PBS to 1 million/ml. 100,000
1176 cells per sample were incubated on ice for 20 min with IgM-PE isotype control (eBioscience
1177 12-4752- 82), TRA-1-60-PE (eBioscience 12-8863-82, PE) or Tra-1-81-PE (eBioscience
1178 12-8883-80) at 1/20 dilution. For propidium iodide (PI) staining, 100µl 1.5µM PI (Sigma)
1179 was added to 100µl cells and incubated for 10-15min on ice. Samples were pelleted and

1180 resuspended in 150 μ l PBS alone (for PI stained samples) or PBS containing 50nM
1181 TOPRO3 (Invitrogen), a dead cell stain, for IgM and TRA-1-60 samples. Samples were run
1182 on the Accuri Cflow flow cytometer. Debris, TOPRO3 positive dead cells (except
1183 when deliberately analyzing dead populations) and cell doublets were excluded from the
1184 analysis.

1185

1186 **Immunostaining.**

1187 For live PI staining, 100 μ l 1.5 μ M PI (Sigma) in PBS was added to wells containing 100 μ l
1188 cells/media. After 10min PI/media was aspirated and replaced with 100 μ l 1:10000
1189 HOESCHT in PBS. Plates were imaged live on the Image Express Micro platform
1190 (Molecular Devices Corporation). For immunostaining, cells were fixed with 4% PFA for
1191 20min, washed with PBS-/- and blocked with 5% NDS in 0.3% triton-X/PBS for 1 hour.
1192 Primary antibody in 1% NDS/TX/PBS was then applied for a further hour at room
1193 temperature. The antibodies used were TRA-1-60 at 1:100 (Abcam 16288, mouse),
1194 PMEL17 at 1:25 (DAKO HMB45, mouse monoclonal) or 1:500 Ki67 (Vector K451, rabbit).
1195 Wells were washed three times with 1% NDS/TX/PBS and secondary antibody, at 1:200
1196 in 1% NDS/TX/PBS applied for 1 hour at room temperature. For TRA-1-60 Jackson anti-
1197 mouse IgM (715-485-020) was used, for PMEL17 Invitrogen Alexa Fluor 488 anti-mouse
1198 and for Ki67 Alexa Fluor 488 antirabbit was used. Wells were then washed three times
1199 with PBS-/- and 1:10,000 HOESCHT applied. These were imaged on the Image Express
1200 Micro platform (Molecular Devices Corporation); automated image analysis was performed
1201 using MetaExpress software. The best settings for this analysis were determined both by
1202 visual observation of the plates and by choosing.

1203

1204 **The introducer tool.**

1205 The patch delivery tool was custom designed and manufactured with the aim of providing
1206 protected delivery of the therapeutic patch until its placement into the subretinal space. The
1207 device consists of a handle containing a mechanism to advance a flexible rod that passes
1208 from the wheel, through the shaft and to the tip where the rod physically pushes the patch
1209 out of the tip of the device (supplementary figure 1.). The rod is advanced by virtue of the
1210 wheel on the handle that the surgeon rolls forward to advance the patch out of the device
1211 and into position.

1212

1213

1214 **Clinical trial.**

1215 Subjects in the trial underwent extensive screening and follow-up investigations including
1216 (i) blood sampling for: Hematology: Hemoglobin, Hematocrit, RBC, Platelet, WBC count
1217 and differential; Chemistry: Urea, Creatinine, Glucose, Calcium, Sodium, Potassium,
1218 Chloride, Total CO₂, AST, ALT, Total Bilirubin, ALP, Uric acid, Albumin, Total protein; HIV,
1219 HepB and HepC testing; (ii) Urinalysis for pH, Glucose, Protein, Blood, Ketones, Nitrites,
1220 Leukocyte esterase, microscopy and a drug screen.

1221

1222 A physical examination, including head, ears, eyes, nose, mouth, skin, heart and lung,
1223 lymph nodes, gastrointestinal, musculoskeletal, and neurological systems, blood pressure,
1224 pulse rate and Electrocardiograms, was also performed.

1225

1226 **Primary endpoints.**

1227 1. Incidence and severity of adverse events.
1228 2. Change from baseline in Early Treatment Diabetic Retinopathy (ETDRS) best corrected
1229 visual acuity (BCVA) - Proportion of subjects with an improvement of 15 letters or more
1230 at Week 24.

1231

1232 **Inclusion criteria.**

1233 1. Male and /or post-menopausal (defined as at least 12 consecutive months with no
1234 menses without an alternative medical cause) female subjects aged 60 years or above.

1235 2. Diagnosis of wet age-related macular degeneration (AMD) with evidence of good
1236 foveal fixation in the study eye plus at least one of:

1237 • History of sudden decline in vision within 6 weeks of the time of surgery (Day 1)
1238 associated with evidence of an RPE tear extending under the fovea.

1239 • History of sudden decline in vision within 6 weeks of the time of surgery (Day 1)

1240 associated with a sub macular hemorrhage with greatest dimension of 6 disc
1241 diameters.

1242 • Evidence of failure to respond to anti-VEGF treatments as defined by declining
1243 vision ≥ 25 ETDRS letters within 20 weeks prior to surgery (Day 1) despite at least 3
1244 injections of anti-VEGF.

1245 3. BCVA value recorded of 6/36 Snellen equivalent or worse in the study eye during or
1246 within 14 d of the screening period.

1247 4. An informed consent document signed and dated by the subject or a legal

1248 representative.

1249 5. Subjects who are willing and able to comply with all scheduled visits, treatment plan,

1250 laboratory tests, and other study procedures.

1251

1252 **Exclusion criteria.**

1253 1. Evidence or history of clinically significant hematological, renal, endocrine, pulmonary,

1254 gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, allergic disease

1255 (including drug allergies, but excluding untreated, asymptomatic, seasonal allergies at

1256 time of dosing) or other severe acute or chronic medical or surgical condition or

1257 laboratory abnormality that may increase the risk associated with study participation or

1258 investigational product administration or may interfere with the interpretation of study

1259 results and, in the judgment of the investigator, would make the subject inappropriate for

1260 entry into this study.

1261 2. Pregnant females; breastfeeding females; and females of childbearing potential

1262 3. Positive urine drug screen for illicit drugs or drugs of abuse.

1263 4. History of regular alcohol consumption exceeding 14 units/week for females or 21

1264 units/week for males within 6 months of screening.

1265 5. Treatment with an investigational drug within 30 d (or as determined by the local

1266 requirement, whichever is longer) or 5 half-lives preceding the first dose of study

1267 medication.

1268 6. Blood donation of >1 pint (500 mL) within 56 d prior to dosing.

1269 7. Subject unwilling or unable to comply with the Lifestyle Guidelines described in this

1270 protocol.

1271 8. Contraindication to general anesthesia (as determined by the attending anesthetist).

1272 9. Previous significant retinal disease in the study eye (other than AMD), as determined by

1273 the investigator.

1274 10. Current or previous significant other ocular disease in the study eye, as determined by

1275 the investigator.

1276 11. Any ocular disease in the study eye that might alter the ocular media and reduce the

1277 posterior segment view, as determined by the investigator.

1278 12. Any previous retinal surgery in the study eye.

1279 13. Contraindication to prednisolone, triamcinolone, cefuroxime or fluocinolone acetonide

1280 (specifically increased intra-ocular pressure or glaucoma).

1281 14. History of sensitivity to heparin or heparin-induced thrombocytopenia.

1282 15. Use of anti-VEGF therapy (e.g., Lucentis) within 7 d of surgery in the study eye.
1283 16. Participation in another ongoing interventional clinical study.
1284 17. Limited use of non-prescription medications that are not believed to affect subject
1285 safety or the overall results of the study may be permitted on a case-by-case basis
1286 following approval by the sponsor.
1287 18. Subjects who are investigational site staff members or relatives of those site staff
1288 members or subjects who are Pfizer employees directly involved in the conduct of the
1289 study.

1290

1291 **Visit schedule.**

1292 Subjects attended the hospital on an out-patient basis for up to three d during the
1293 screening interval (Day -21 to Day 0). Eligible subjects attended the hospital on an
1294 out-patient basis the day before surgery (Day 0) for pre-surgery assessments. Insertion of
1295 PF-05206388 (the hESC-RPE patch) occurred the following day (Day 1). Subjects were
1296 discharged from the hospital the day after surgery (Day 2). There was a second operative
1297 procedure performed at the Week 10 visit (Visit 9) whereby the silicone oil, which was
1298 inserted at the time of PF-05206388 insertion, was removed from the eye. Subjects with
1299 significant cataracts noted at screening were permitted to have the cataracts removed at
1300 the time of this second surgical procedure. For the first procedure, subjects received up to
1301 1 mg/kg oral prednisolone (up to 60 mg maximum) daily commenced 2-4 d pre-operatively
1302 and continued for at least 2 weeks followed by a tailing off over at least 1 further week.
1303 Subjects also received single doses of sub-Tenon triamcinolone acetonide 40 mg and sub-
1304 conjunctival cefuroxime (250 mg in 1 mL) after patch insertion. For the second procedure,
1305 subjects received up to 1 mg/kg oral prednisolone (up to 60 mg maximum) daily
1306 commenced 7 d pre-operatively and continued for at least 2 weeks followed by a tailing off
1307 over at least 1 further week. Subjects also received an intravitreal implant of fluocinolone
1308 acetonide either 0.19 or 0.59 mg as an anti-inflammatory agent and sub conjunctival
1309 cefuroxime (250 mg in 1 mL) post-surgery.

1310

1311 The first surgical procedure was carried out on Day 1, subjects were discharged from
1312 hospital on Day 2 and attended for post-surgical evaluations at Week 1, Week 2, Week 4,
1313 Week 8, Week 12, Week 16, Week 24, Week 36 and Week 52.

1314

1315

1316 **Surgical procedures.**

1317 Transplantation operation: patients underwent standard general anaesthesia. Routine sterile
1318 povidone-iodine 5% preparation of the eye with and draping of the face was completed.
1319 The eyelids were separated with a disposable Barraquer speculum. For case 1 a cataract
1320 was removed and a single focus, one-piece, acrylic lens for emmetropia was inserted, at
1321 the time of the transplant surgery, while subject 2 was already pseudophakic. A 270 degree
1322 peritomy with a standard 23-gauge infusion and 2 further ports were inserted. Pars plana
1323 vitrectomy with induction of PVD when not present was completed with subsequent 360
1324 degree Argon laser. A macular retinal detachment was created with a 38 gauge de Juan
1325 dual bore cannula, the retinotomy was enlarged with microscissors and the subretinal
1326 space irrigated to remove all blood and fibrin. The temporal port was widened for
1327 introduction of the delivery device and insertion of the patch into the sub retinal space. The
1328 macula was re-attached with heavy liquid and the retinotomy lasered. A routine search of
1329 the retinal periphery was completed in both cases with no iatrogenic breaks detected.
1330 Air/fluid followed by air/silicone oil exchange was carried out. The ports were sutured and
1331 the infusion removed. Sub-Tenon triamcinolone acetonide 40mg and subconjunctival
1332 cefuroxime (250 mg/1 ml) were injected followed by sub-Tenon (0.5% bupivacaine).

1333

1334 Removal of Silicone Oil: silicone oil was removed under sub-Tenon local anaesthesia. A
1335 23-gauge infusion was inserted in the infero-temporal quadrant and a second port created
1336 to aspirate the oil. The retinal periphery was examined for breaks. The ports were closed
1337 and subconjunctival antibiotics were given. For subject 2 an operation to reattach a
1338 detached retina, consisting of epiretinal membrane peeling, inferior 180 degree retinectomy
1339 and laser, was carried out before the oil removal.

1340

1341 **Ocular tests and examinations.**

1342

1343 Biomicroscopic evaluation and intra-ocular pressure measurement were carried out using a
1344 Haag-Streit 900 slit-lamp (Koeniz, Switzerland) and Goldmann applanation tonometer.

1345

1346 **Fundus photography, angiography.**

1347 Colour, red-free fundus photographs and standard fluorescein and indocyanine green
1348 angiography (50°) were acquired through dilated pupils using the Topcon TRC Retinal
1349 Camera (Oakland, NJ, USA).

1350

1351 **Spectral-domain OCT scan and cSLO reflectance imaging.**

1352 Volume scan and EDI volume scan, 20°x20°, 49 sections, ART 12, High Speed scans and
1353 30°, 55° short-wave reflectance autofluorescence images were acquired through dilated
1354 pupils using the Spectralis OCT (Heidelberg Engineering GmbH, Heidelberg, Germany)

1355

1356 **Adaptive optics.**

1357 AO images were acquired through dilated pupils using the ImagineEyes Camera (Orsey,
1358 France). Alignment and data collection was repeated for several retinal locations.
1359 For calculation of cellular density, a freely available image-processing program (ImageJ,
1360 National Institutes of Health, Bethesda, Maryland) was used to manually identify the cones
1361 in each subject's retinal image.

1362

1363 **Pattern ERG, full-field ERG and EOG.**

1364 Pattern ERG, full-field ERG and EOG were performed according the ISCEV standards.
1365 Dark-adapted ERGs were recorded to flashes of 0.01 and 10.0 cd.s.m-2 (DA0.01; DA10.0);
1366 light adapted responses were recorded to flashes of 3.0 cd.s.m-2 (30Hz and 2Hz).

1367

1368 **Automated perimetry.**

1369 Visual fields were assessed using Humphrey Field Analyzer (Carl Zeiss Meditec, AG, Jena,
1370 Germany). Central 24-2 Threshold tests were performed using Goldmann III white stimulus
1371 at background luminance of 31.5ASB, in SITA-FAST strategy.

1372

1373 **Microperimetry.**

1374 A customized rectangular grid of 9-23 loci covering the area of the RPE patch was used in
1375 Nidek MP1 Microperimeter (NAVIS software 1.7.2, Nidec Technologies, Padova, Italy).
1376 Stimuli were set to Goldmann III-V size, white color, 200ms duration and 0-20dB intensity
1377 with background luminance at 1.27cd/m². A single red-cross 2-3° target was used for 30
1378 seconds fixation stability assessment. A 4-2 staircase strategy was employed to assess the
1379 retinal sensitivity through dilated pupils after 15min dark adaptation.

1380

1381 **ETDRS/logmar best corrected visual acuity (BCVA) - distant and near,
1382 including contrast sensitivity (CS).**

1383 Refracted BCVA was tested by Independent Trained optometrists using the Early

1384 Treatment of Diabetic Retinopathy Study (ETDRS) charts and CS the Pelli-Robson chart at
1385 1 meter.

1386

1387 **Minnesota reading test (MNREAD).**

1388 The MNREAD sentences were used to assess the visual processing capabilities and eye-
1389 movement control required for normal text reading. Each sentence contains 60 characters
1390 (including a space between each word and at the end of each line) printed as three lines
1391 with even left and right margins. The vocabulary used in the sentences is selected from
1392 high-frequency words that appear in second- and third-grade reading material. The charts
1393 contain sentences with 19 different print sizes. From the recommended viewing distance of
1394 40 cm (16 in.) the print size ranges from +1.3 to -0.5 logMAR (Snellen equivalents: 20/400-
1395 20/6).

1396

1397 **B-mode orbital ultrasound.**

1398 Ultrasound scans were acquired through sterile gel, using the ACUSON SequoiaTM512
1399 system (Siemens Healthcare, USA)

1400

1401 **Ocular oncologist review.**

1402 Specialist Ocular oncologists reviewed the patients with biomicroscopic evaluation and
1403 review of images from the ultrasound, fundus photography and fundus angiography.

1404

1405 **Data validation.**

1406 This manuscript is generated from an unlocked database and includes some data that
1407 Pfizer is not responsible for validating/storing.

1408

1409 **Data availability statement.**

1410 The majority of data generated or analysed during this study are included in this published
1411 article and its supplementary information. Any further data concerning the current study are
1412 available from the corresponding author on reasonable request. There are no accession
1413 codes, unique identifiers, or web links to publicly available datasets related to this study.

1414

1415 **Online Methods References**

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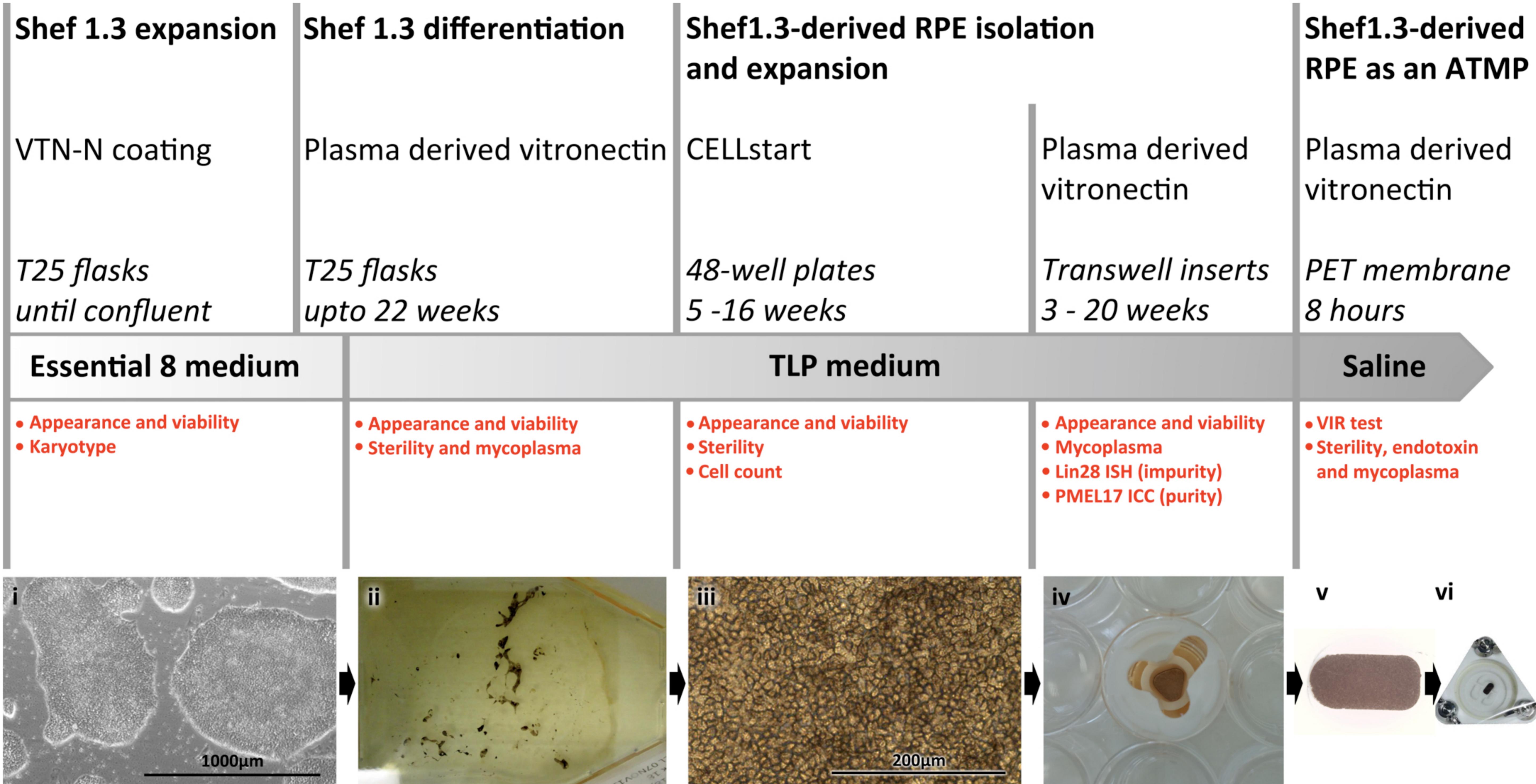
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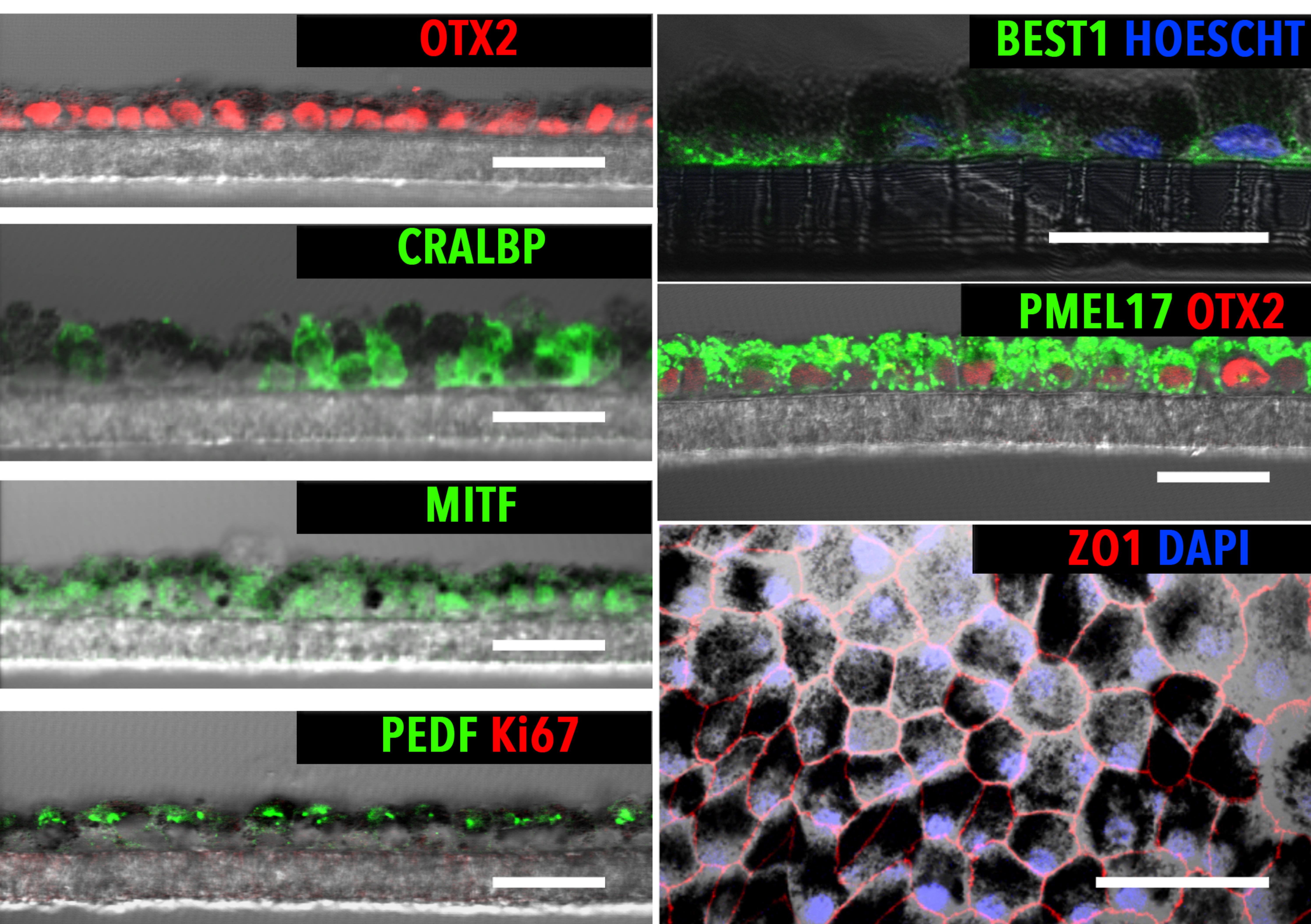
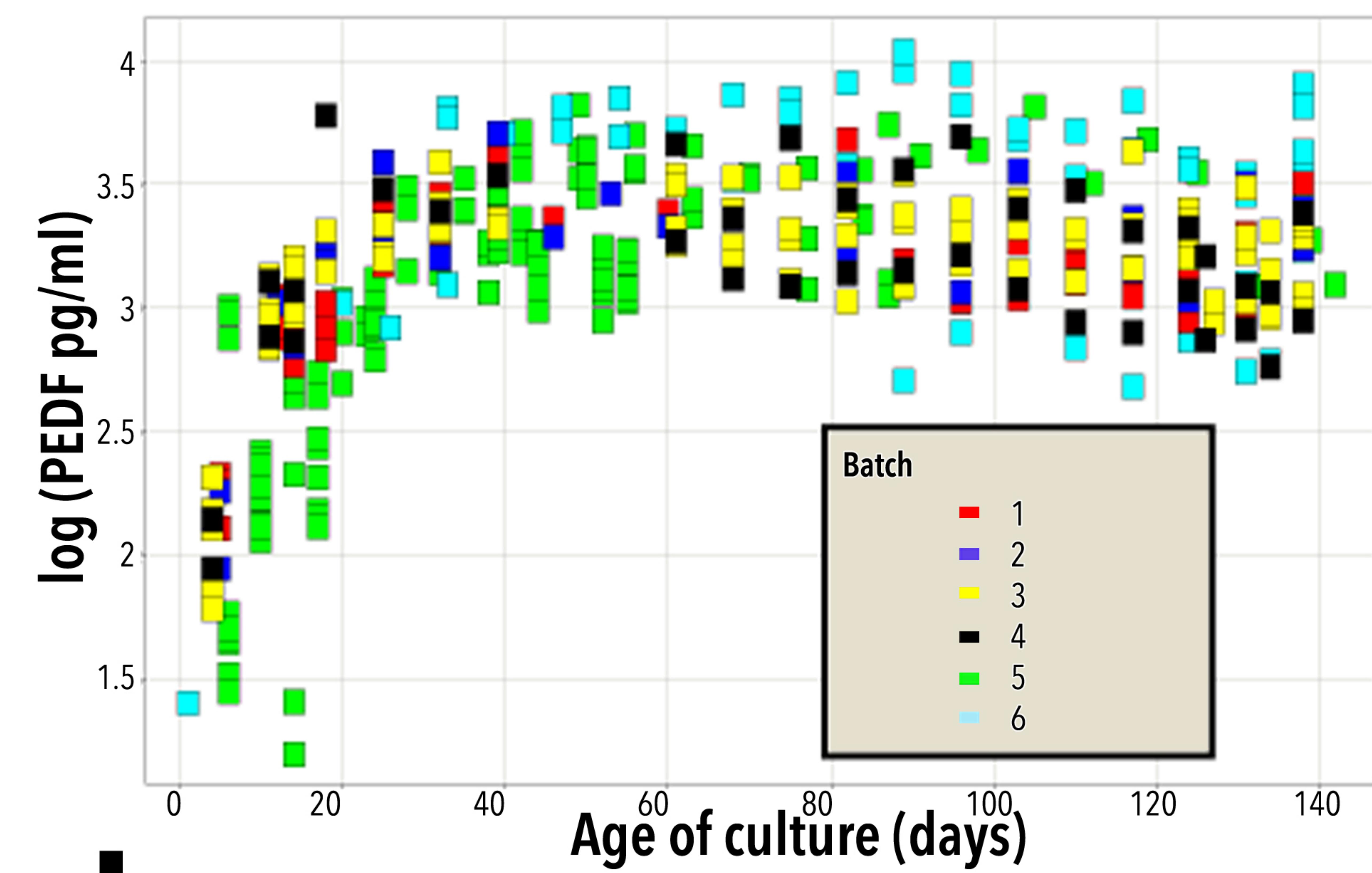
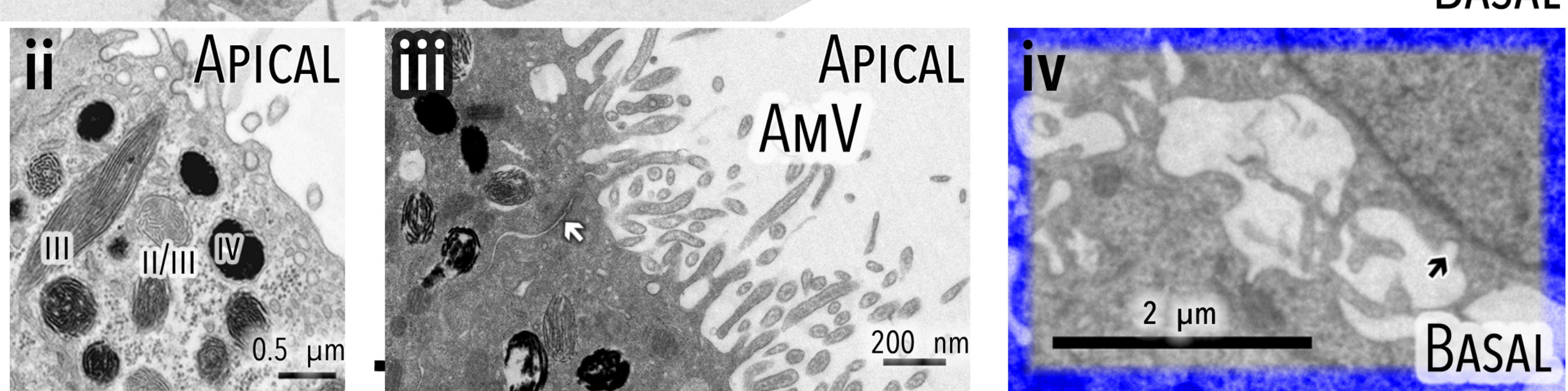
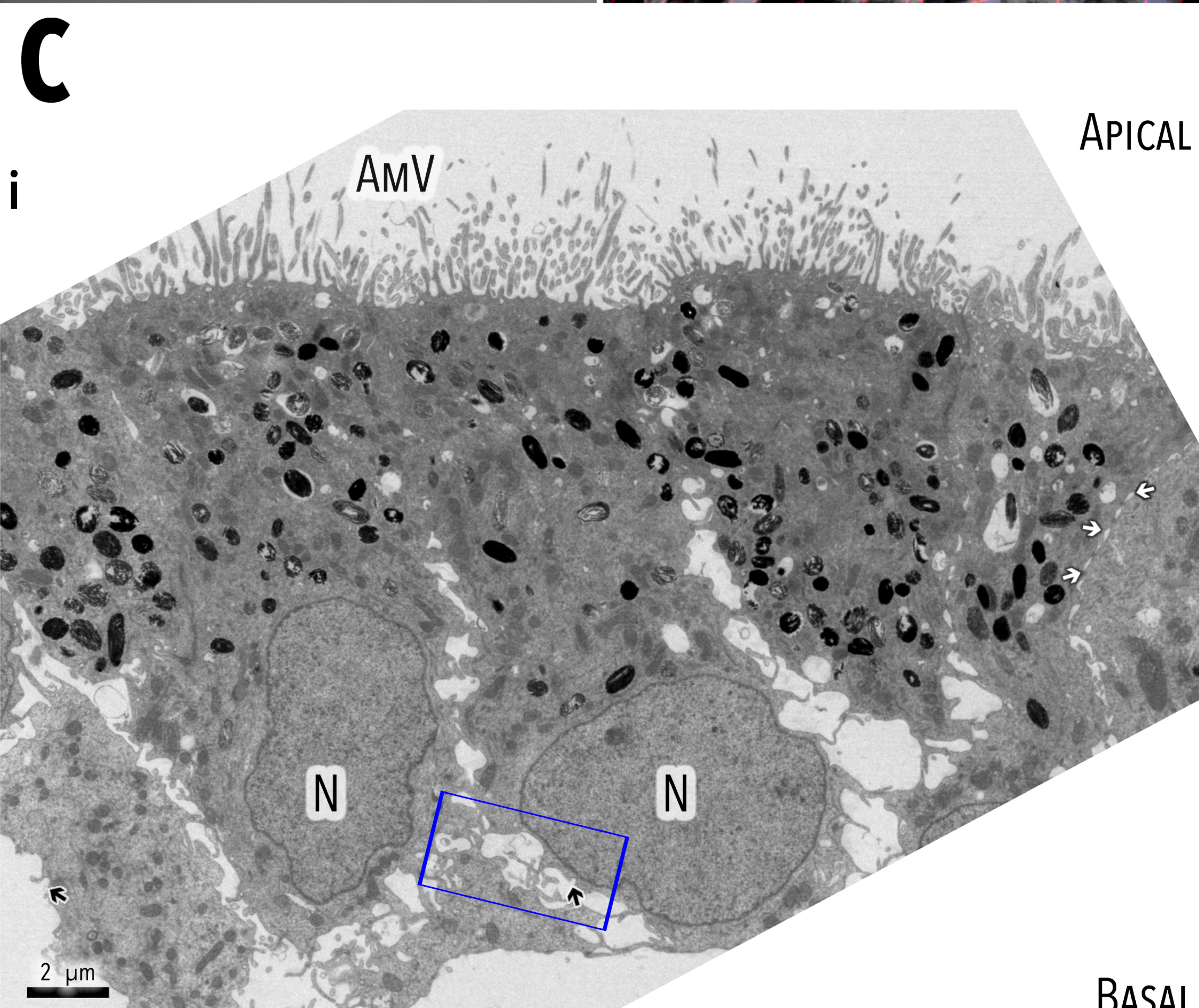
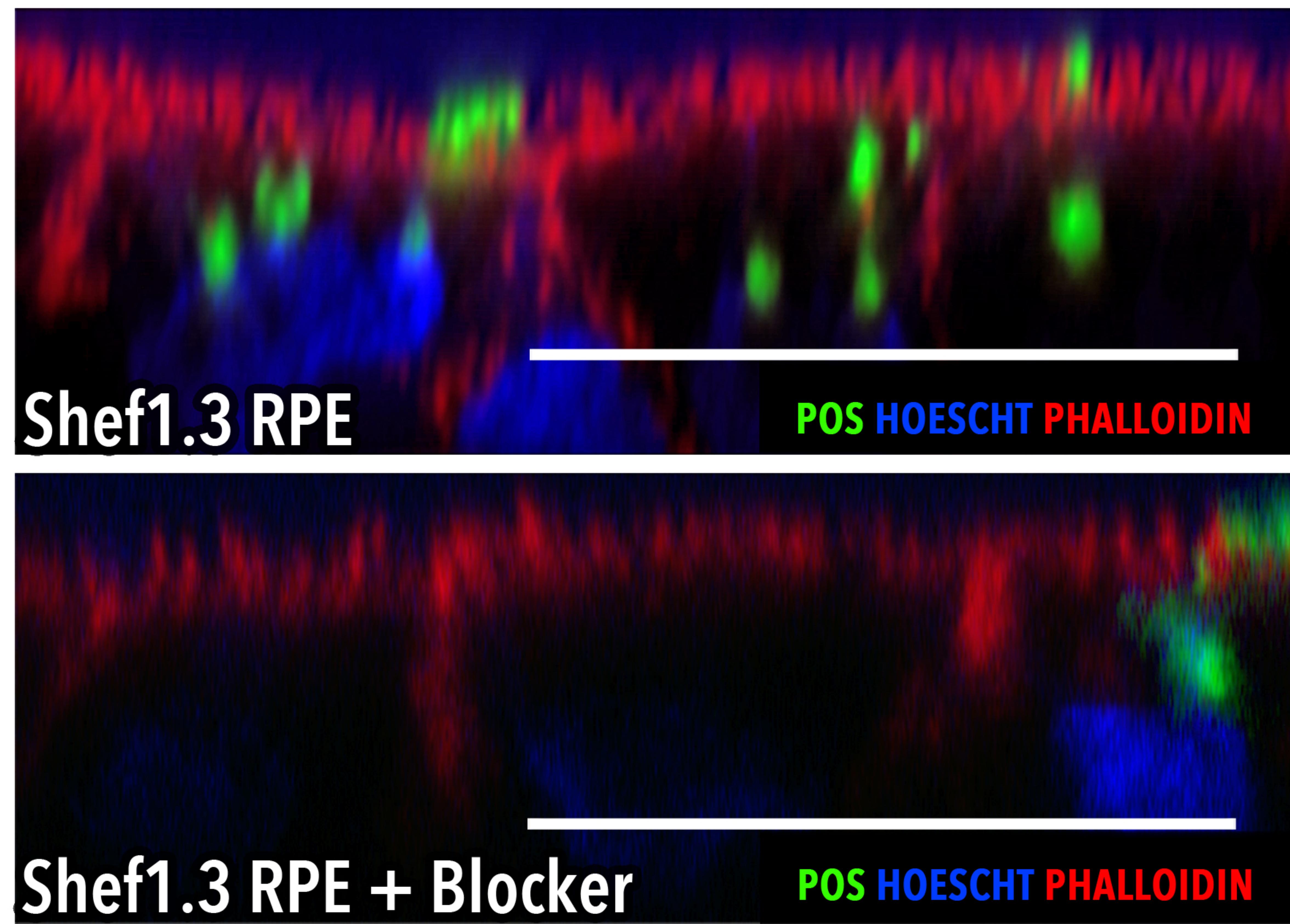
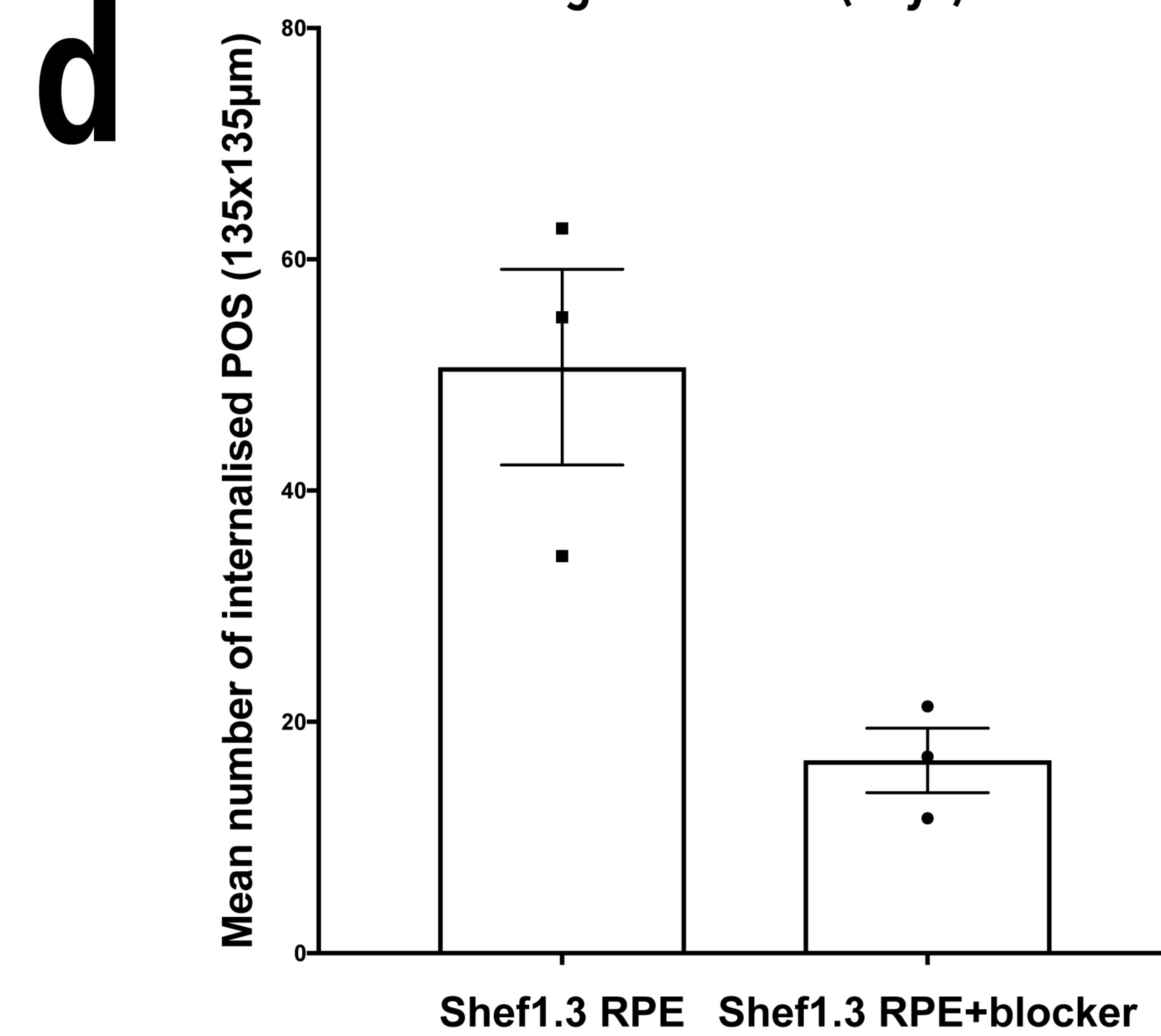
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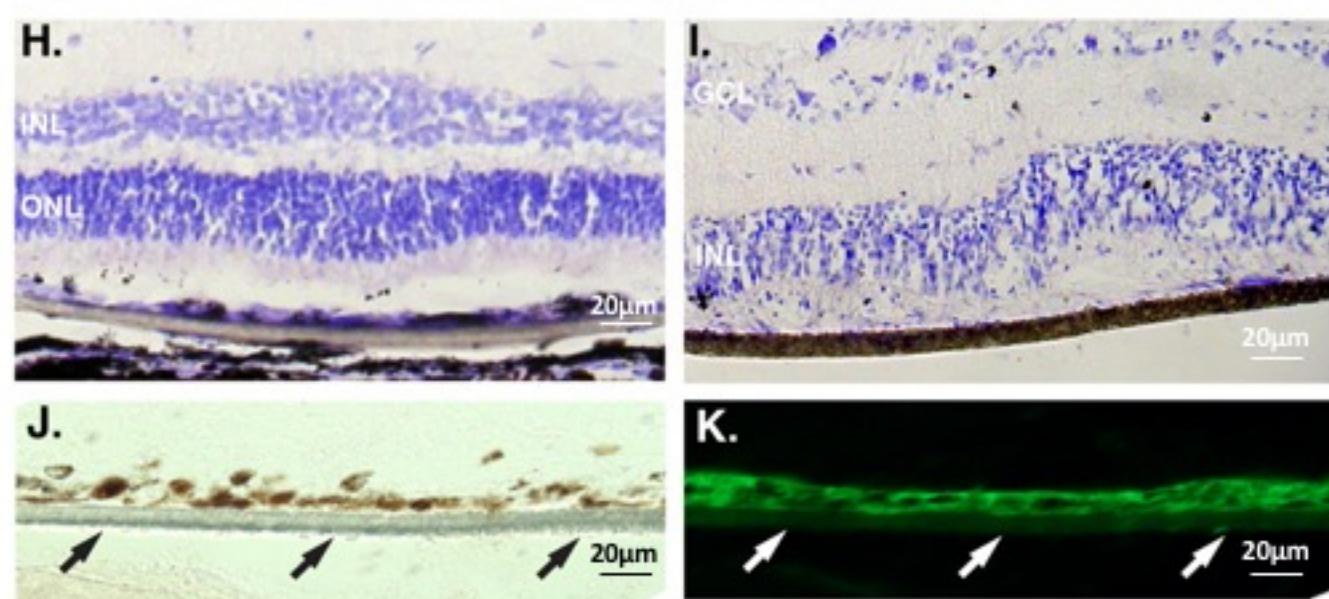
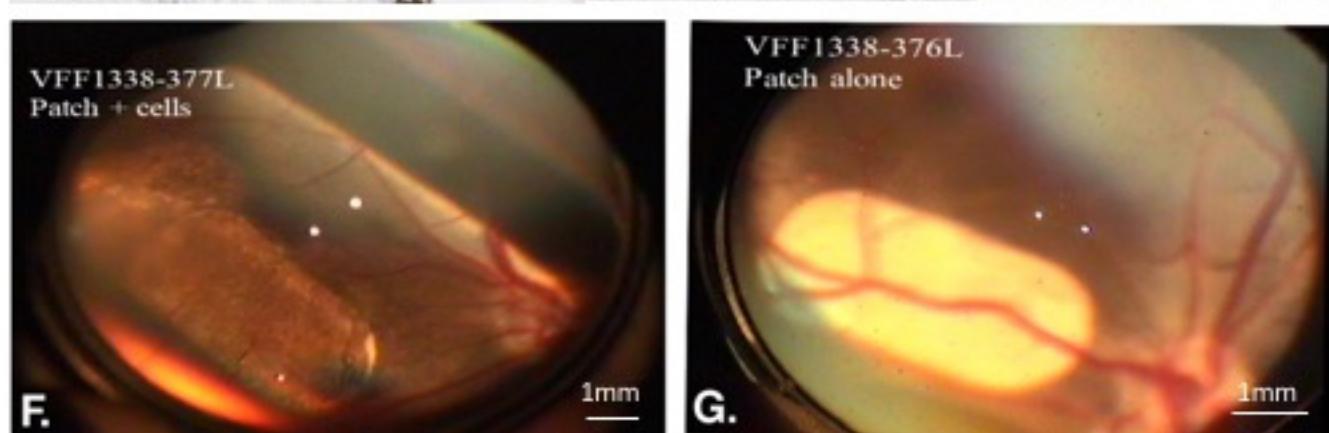
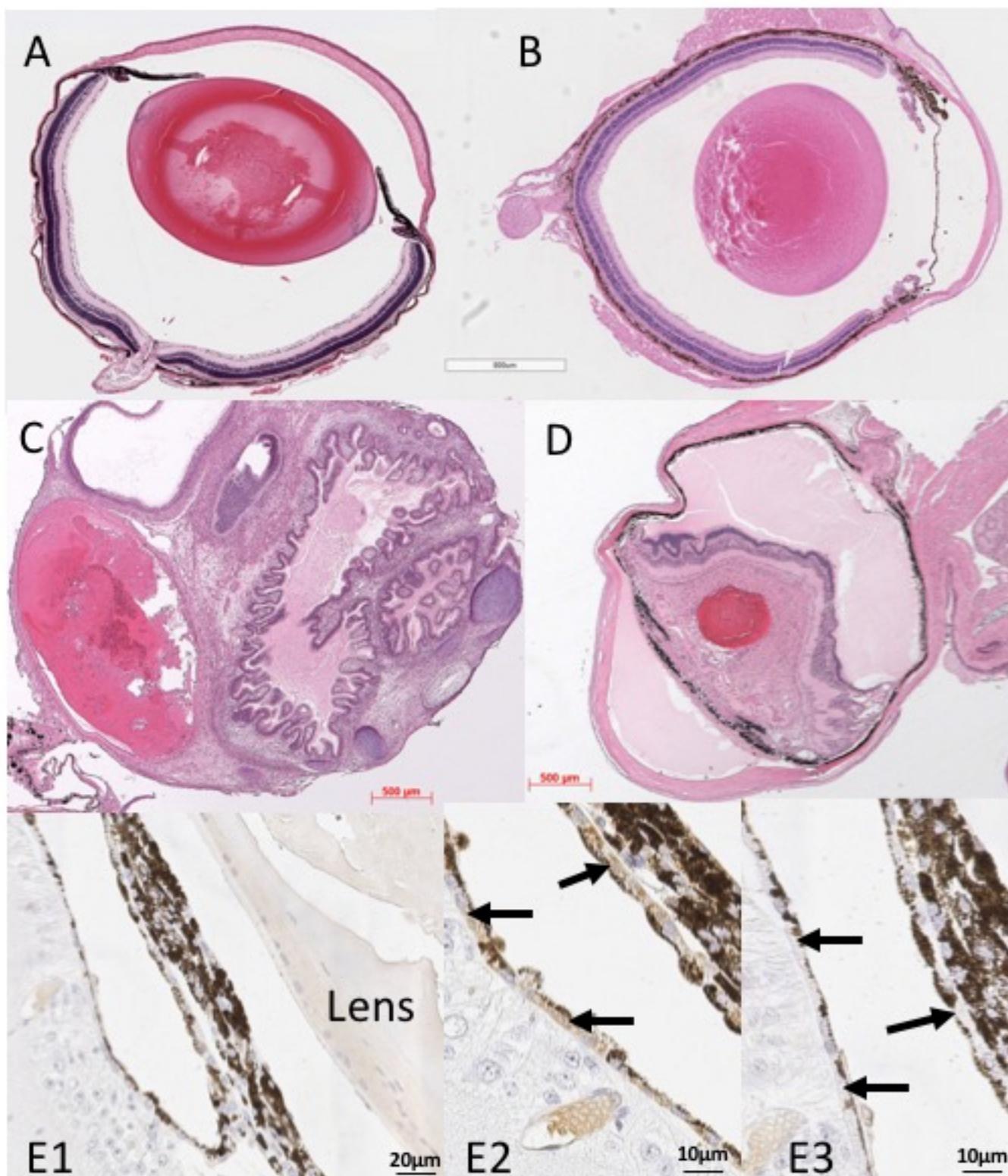
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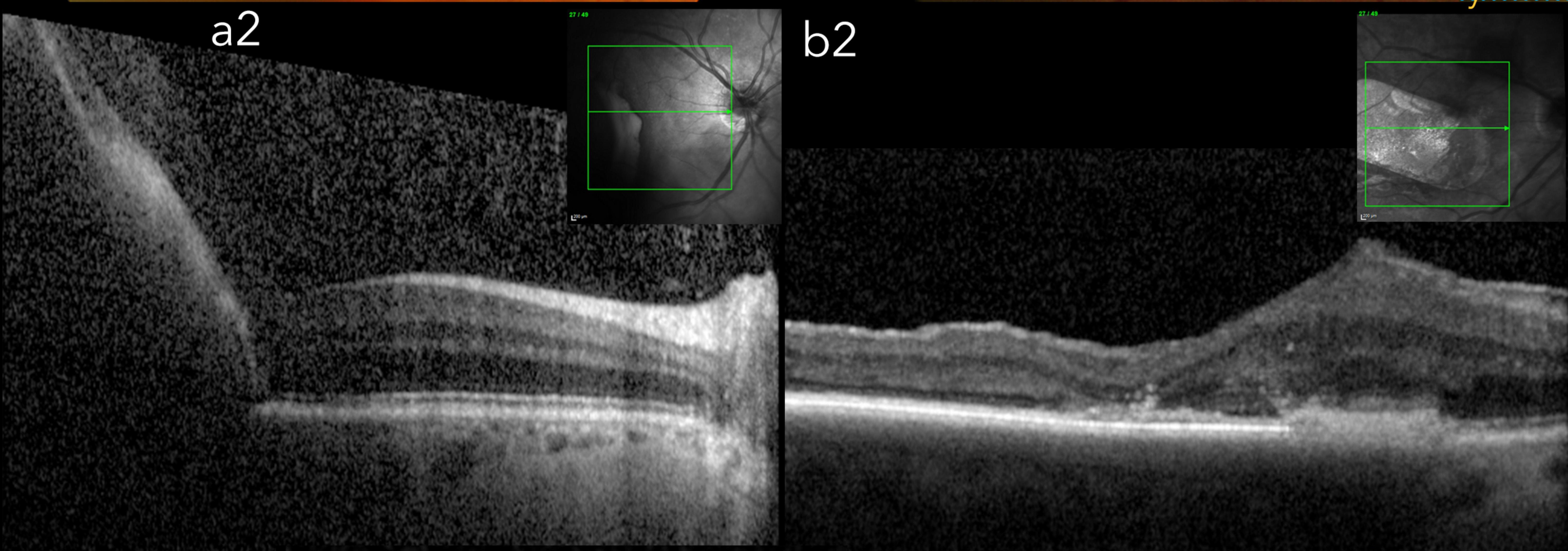
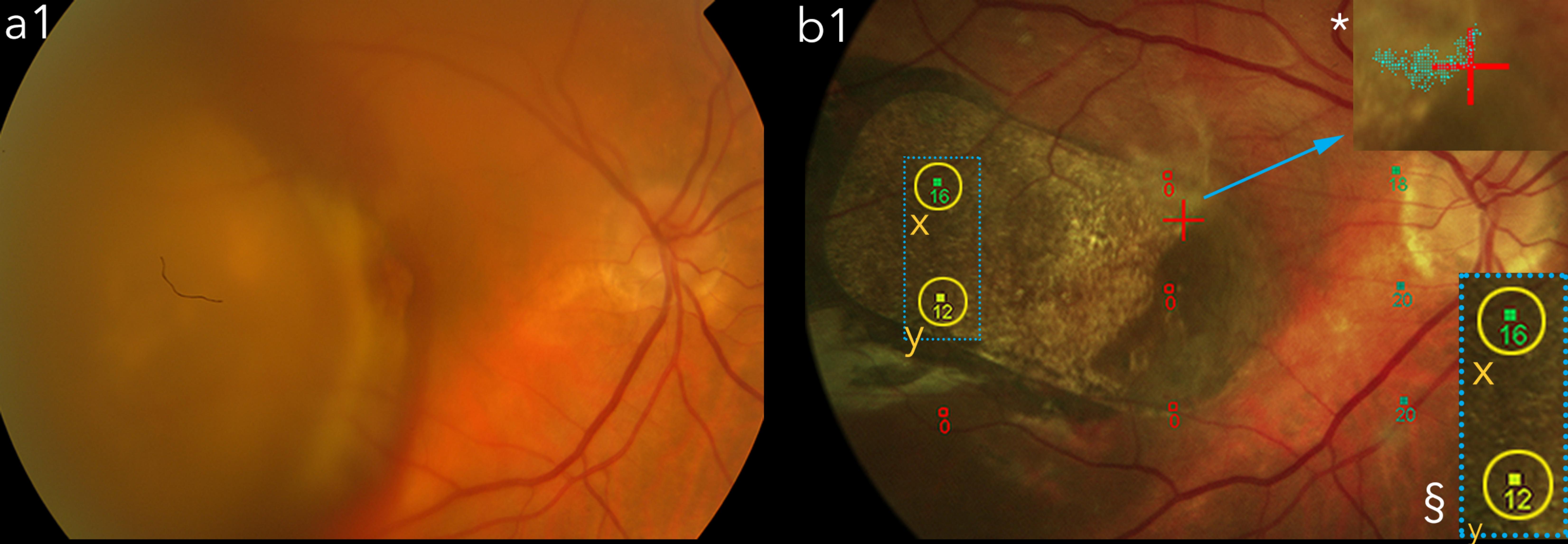
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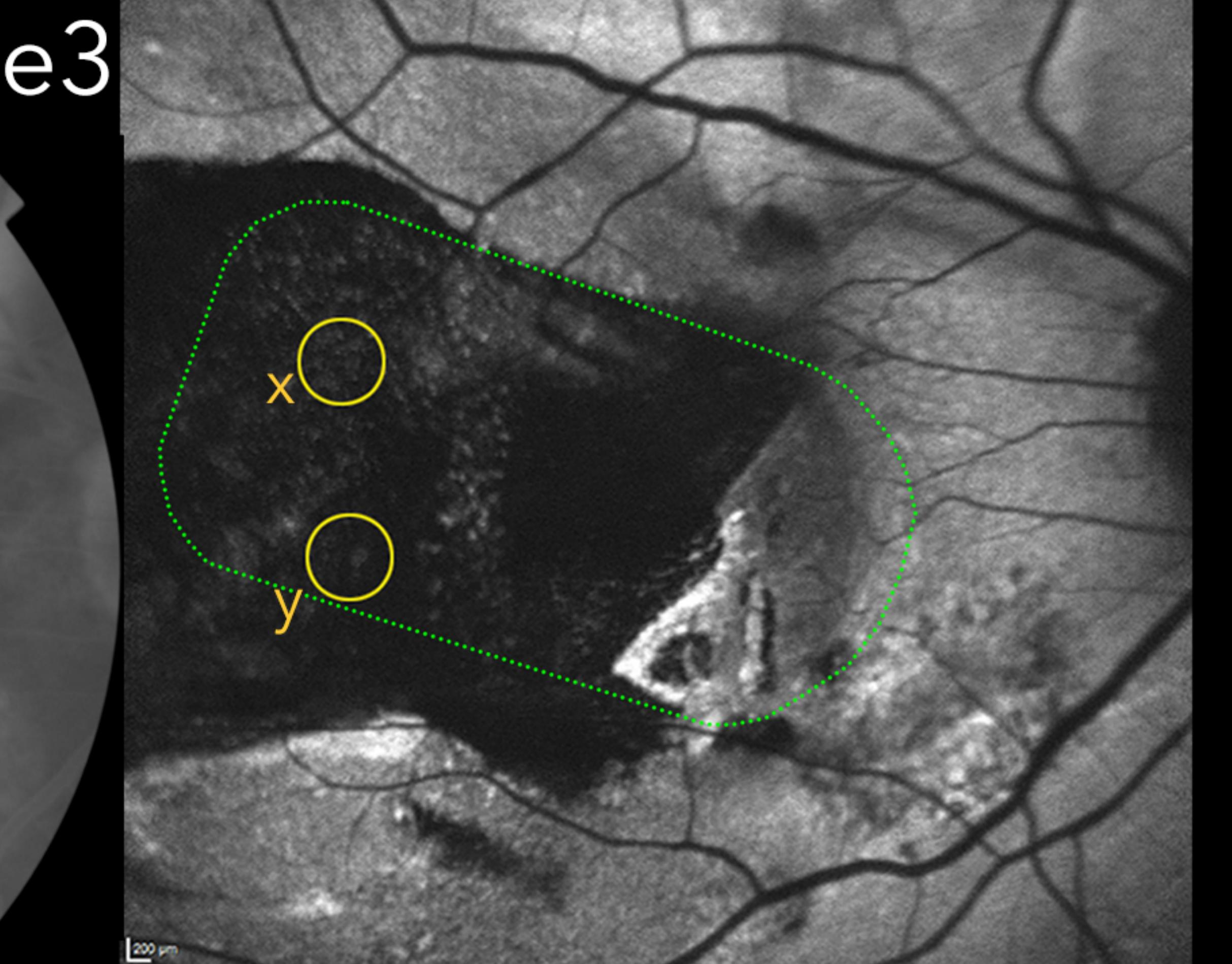
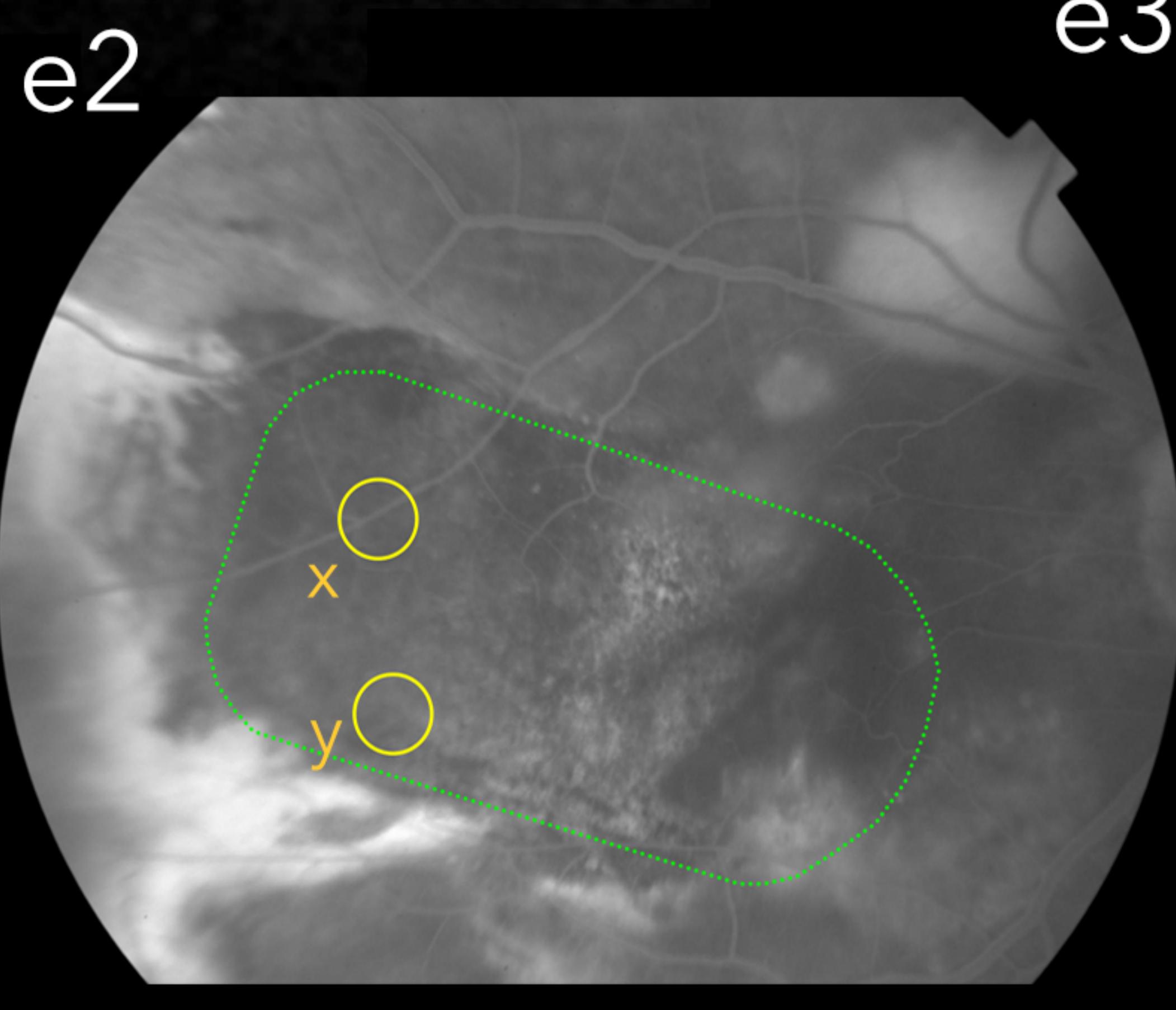
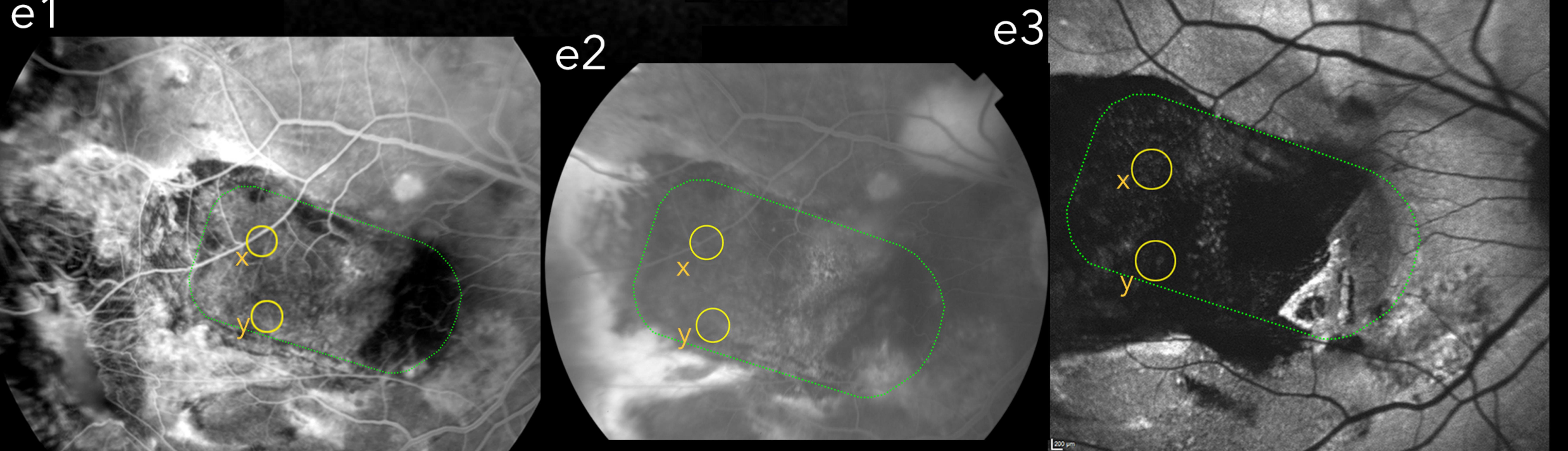
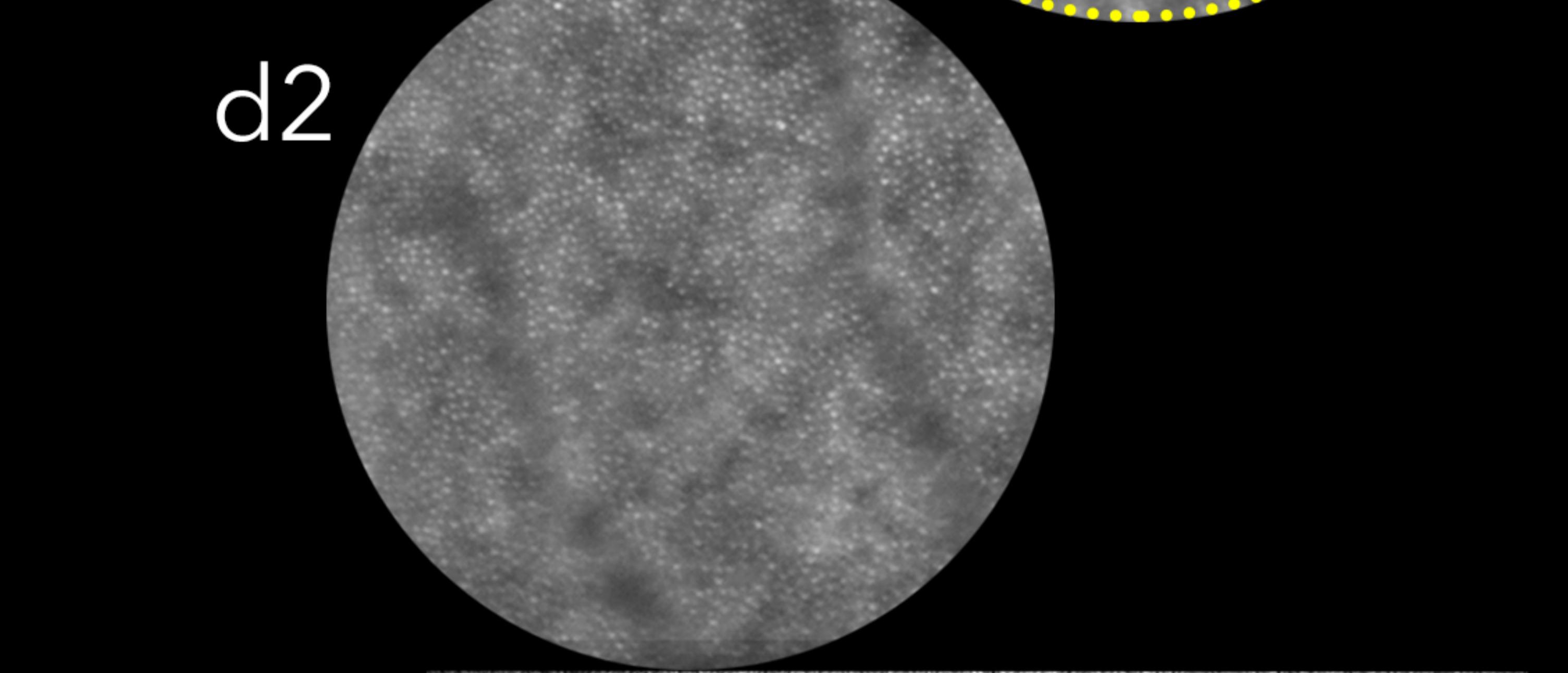
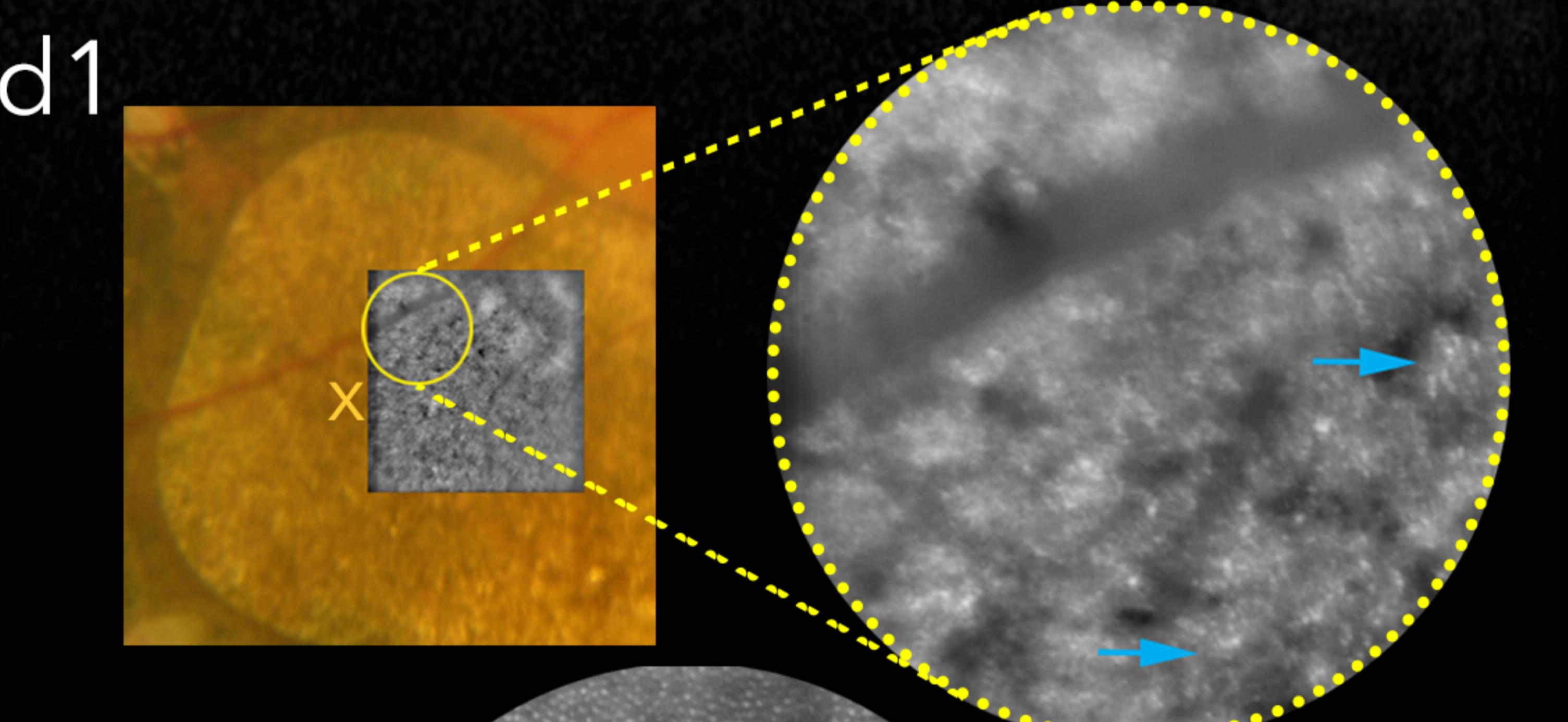
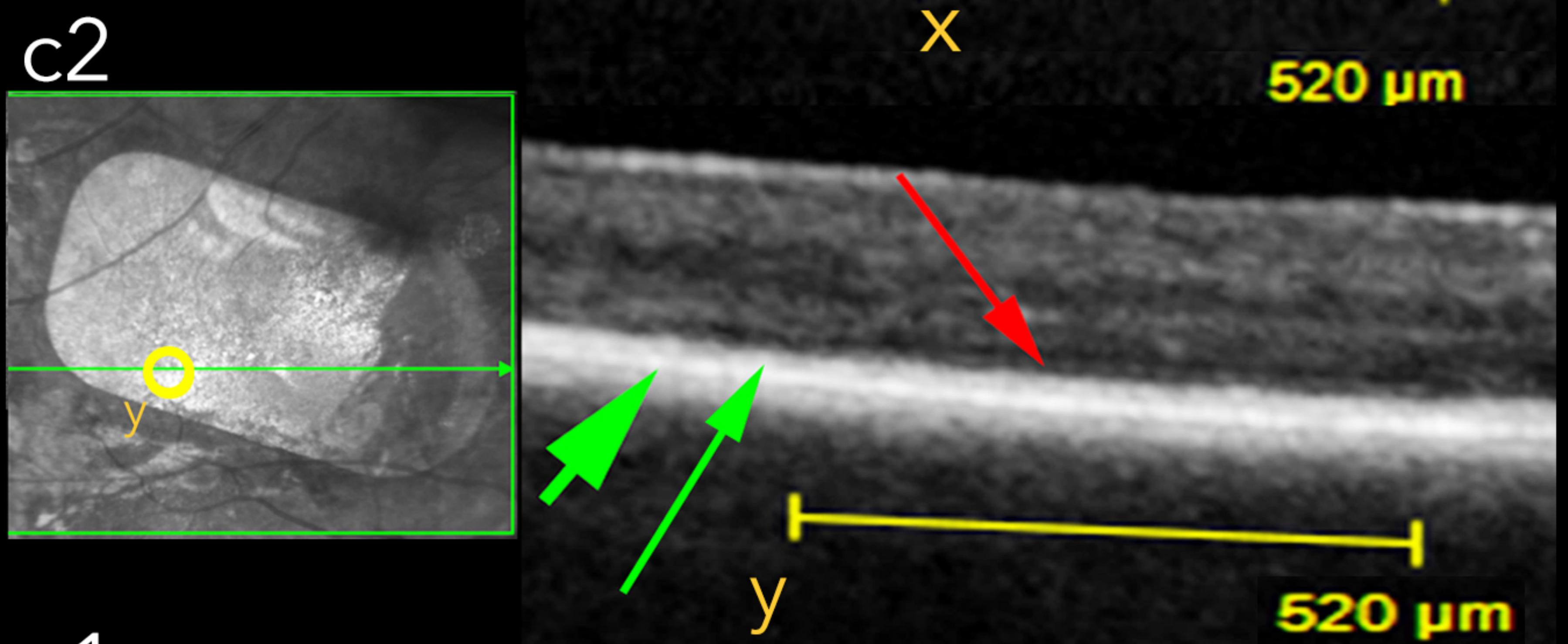
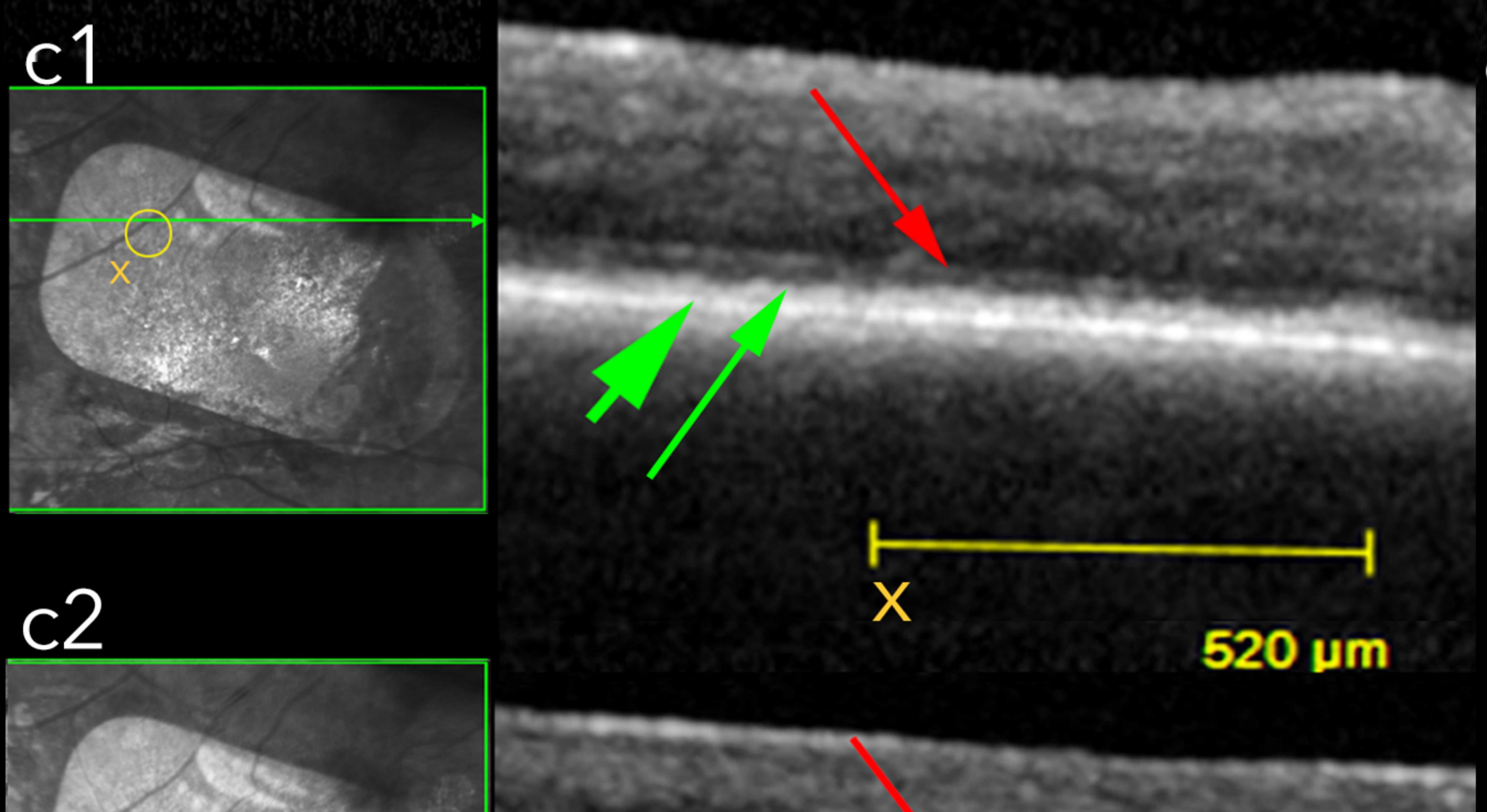
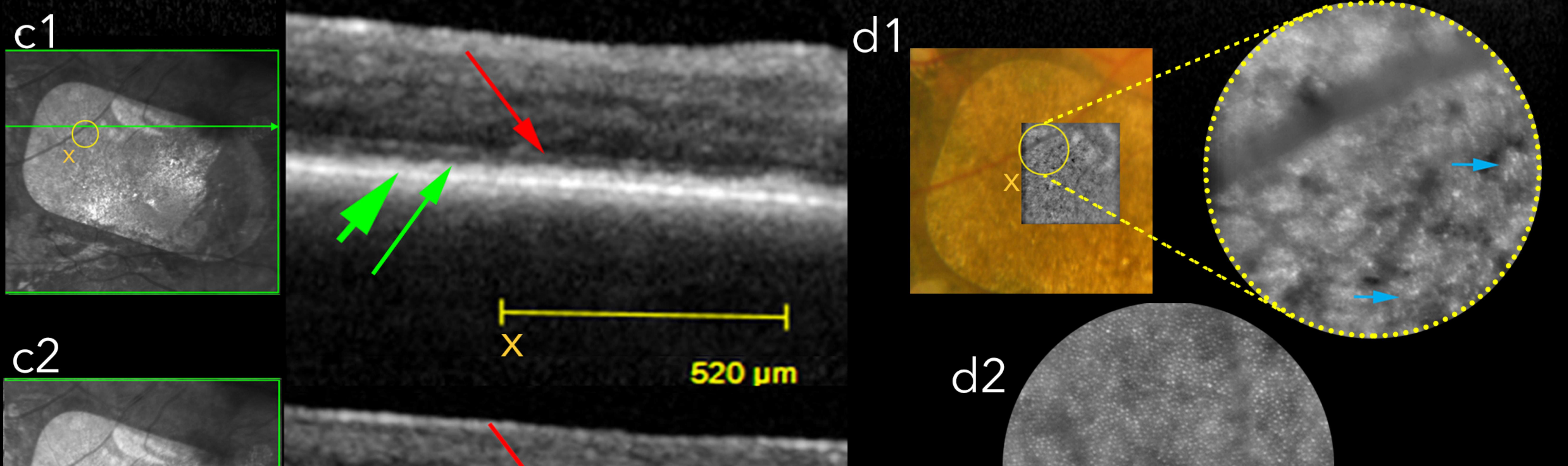


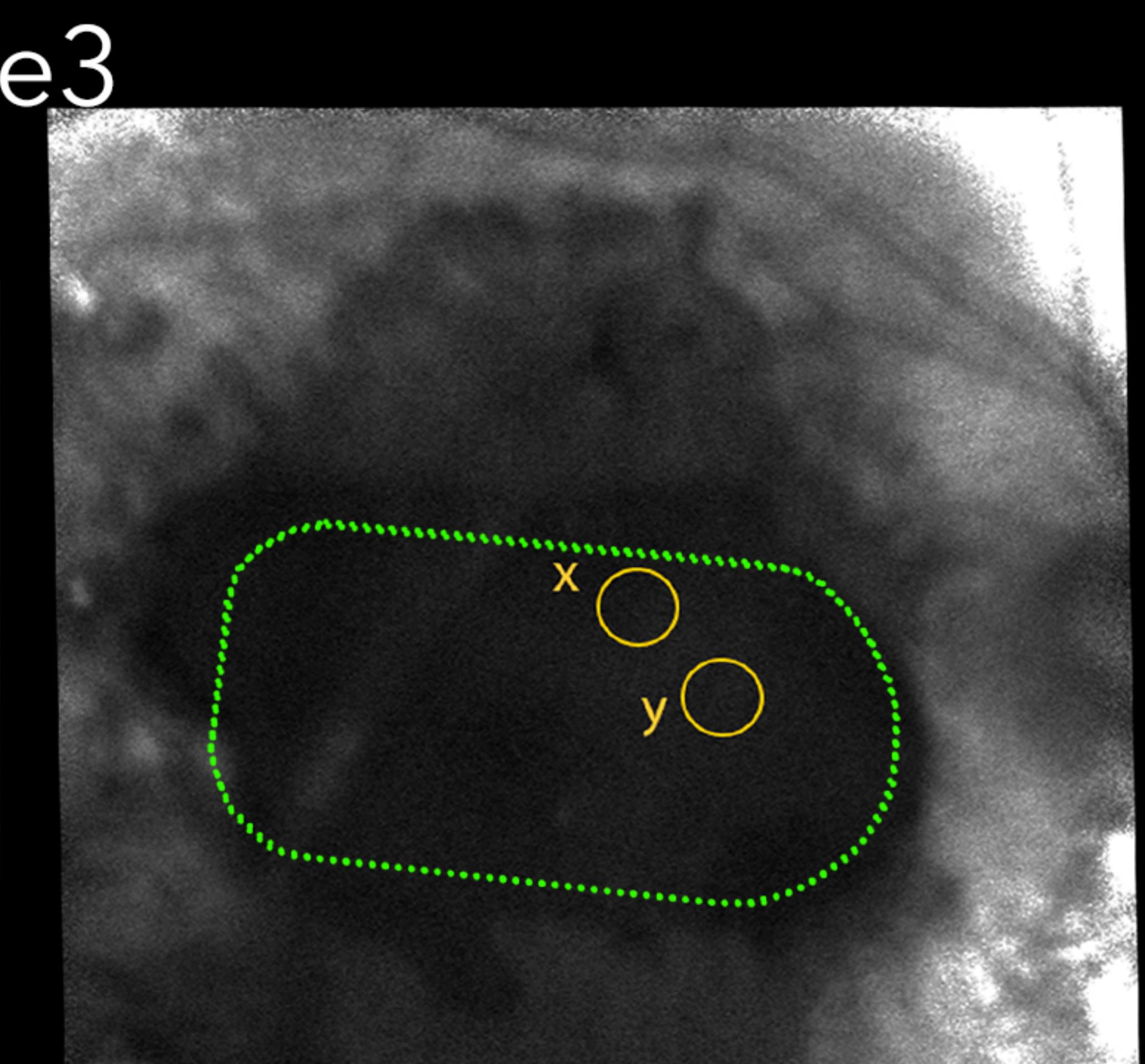
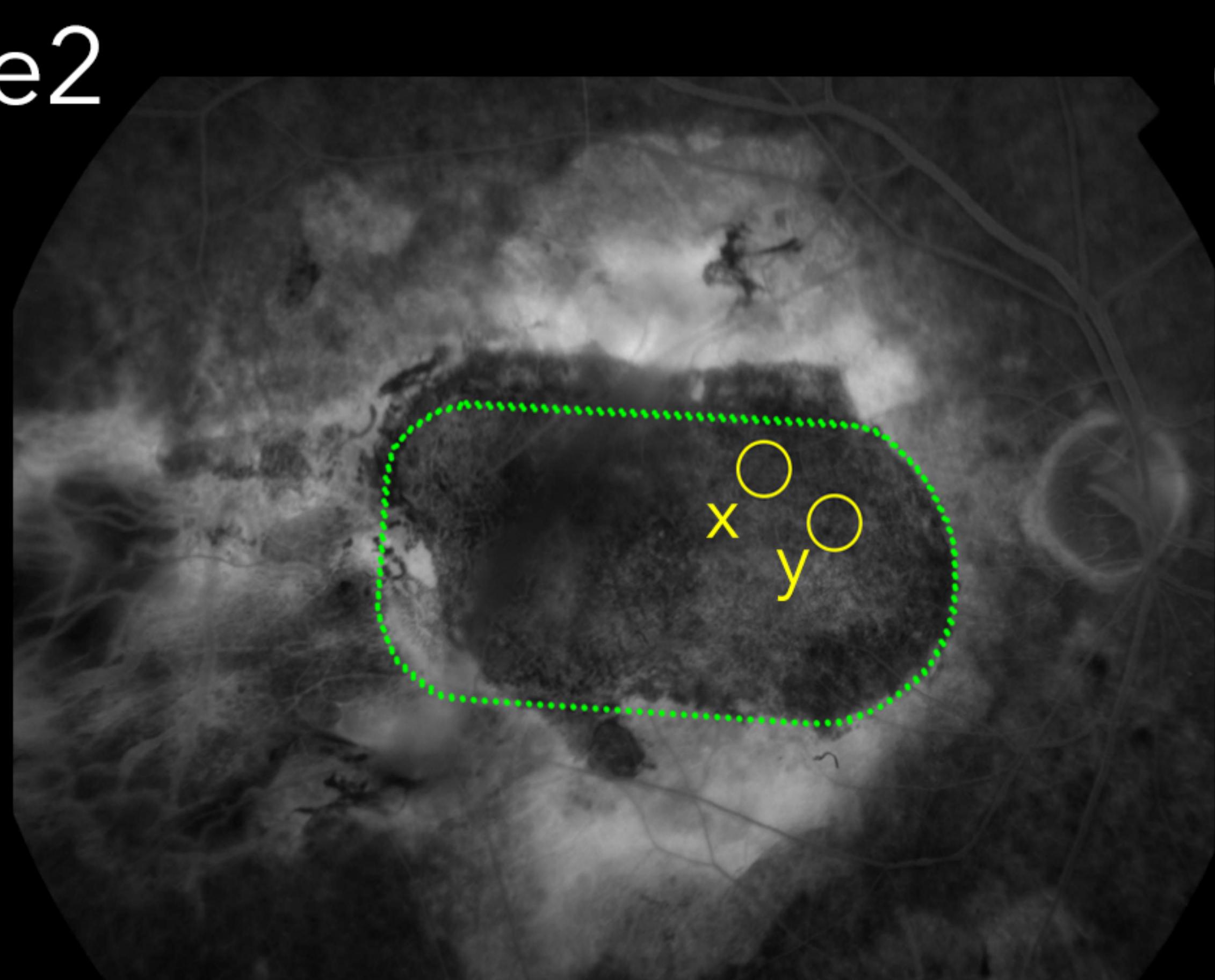
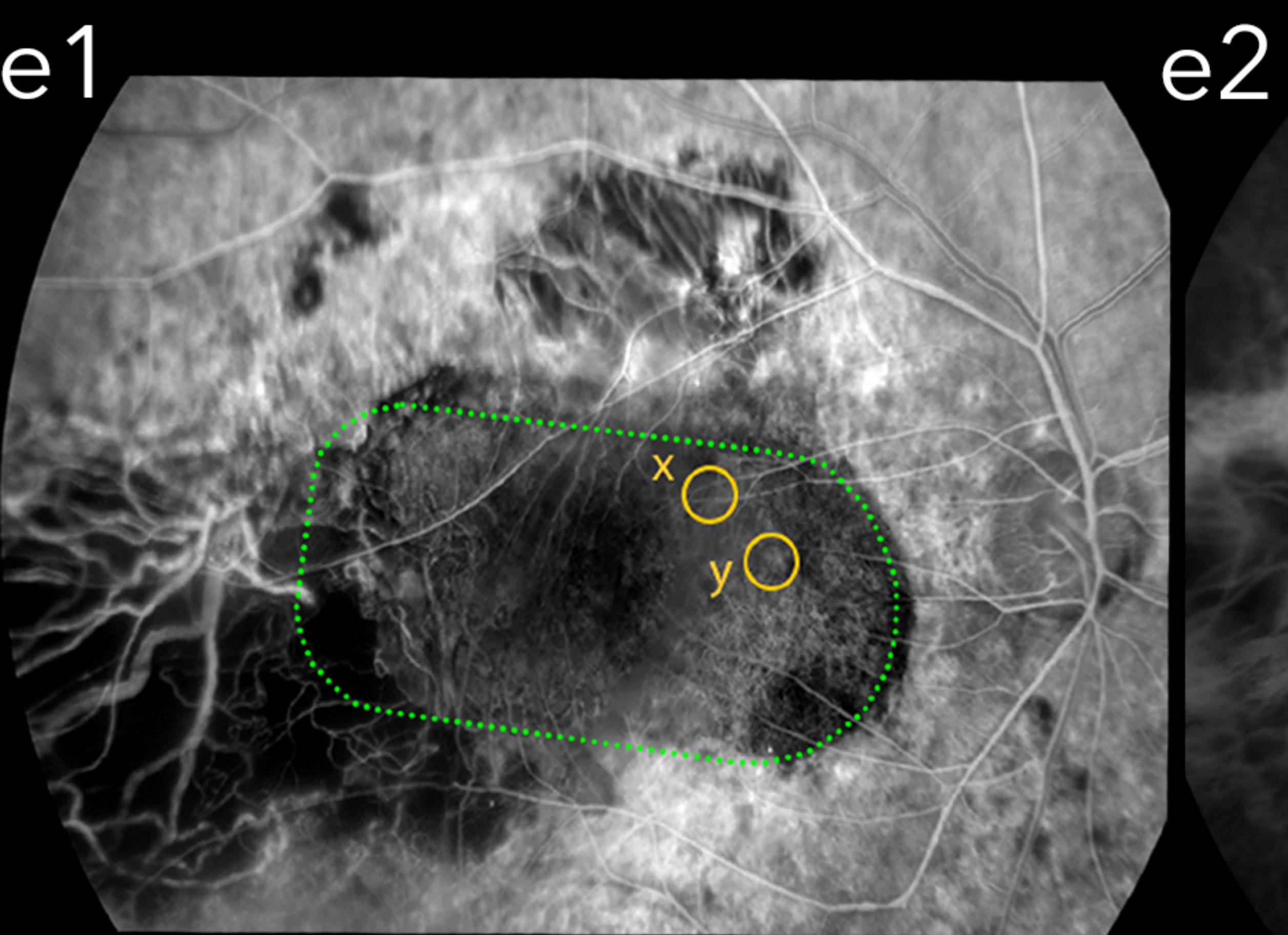
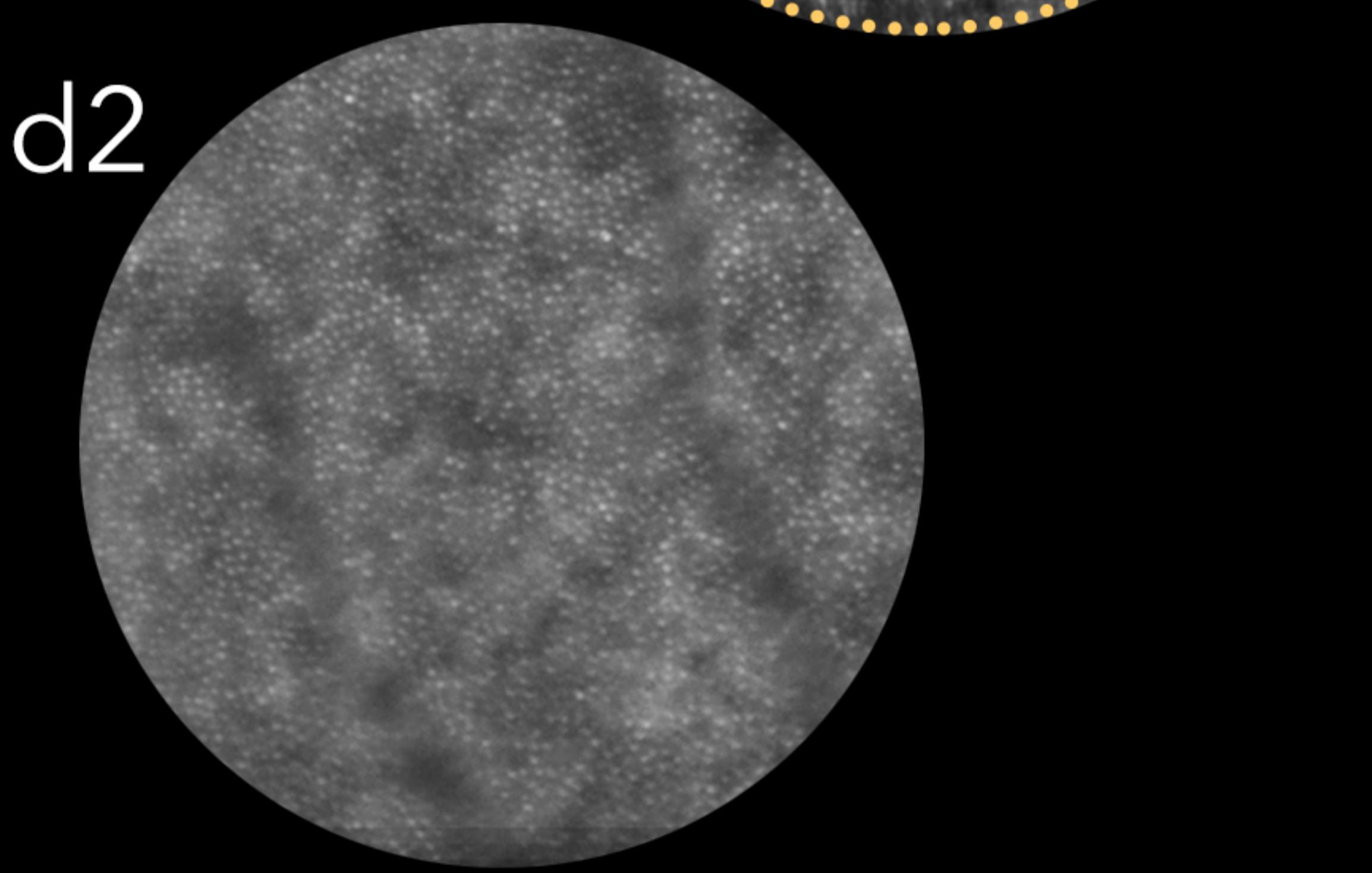
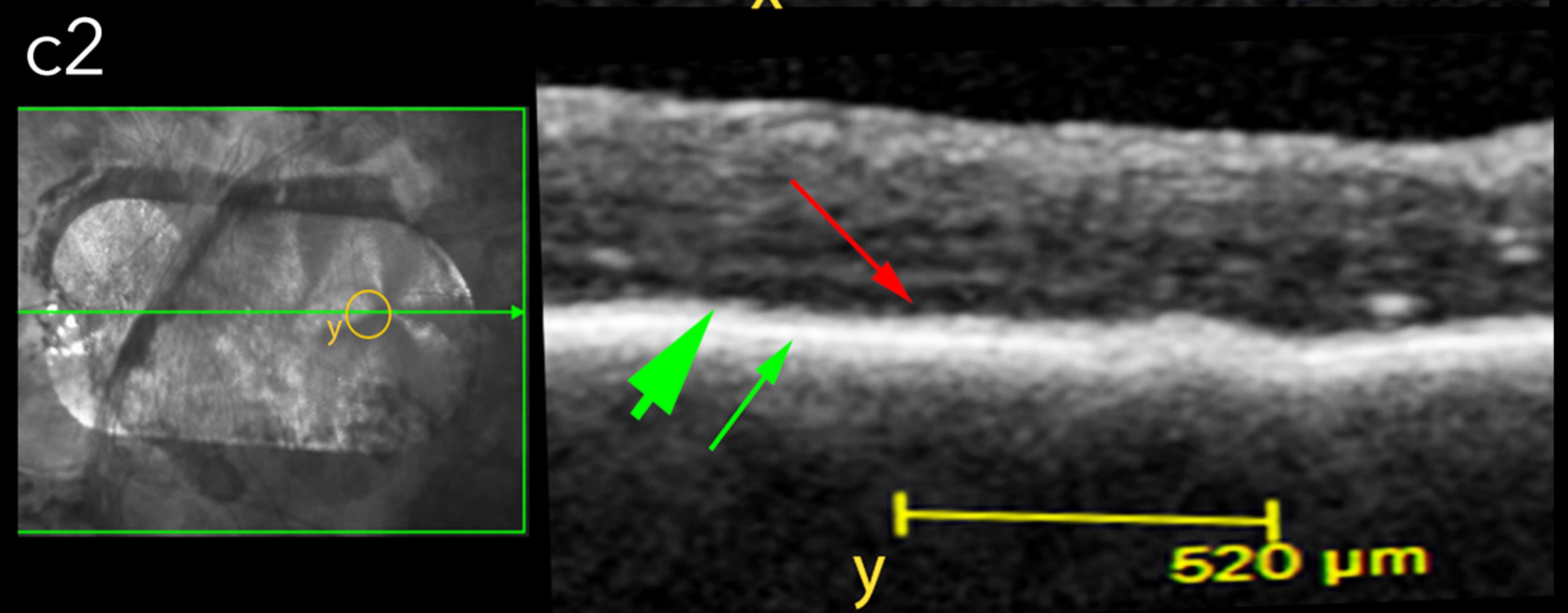
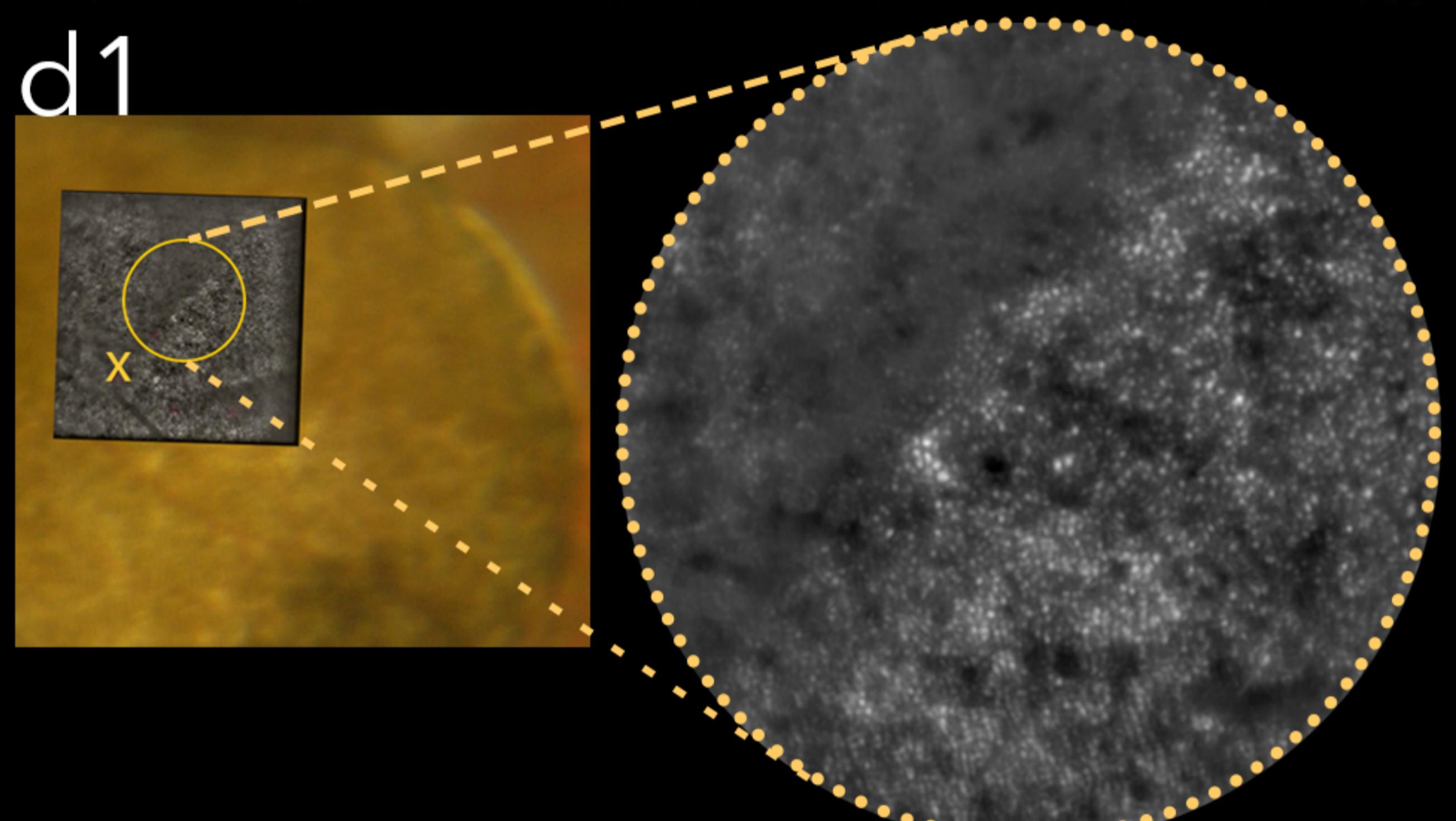
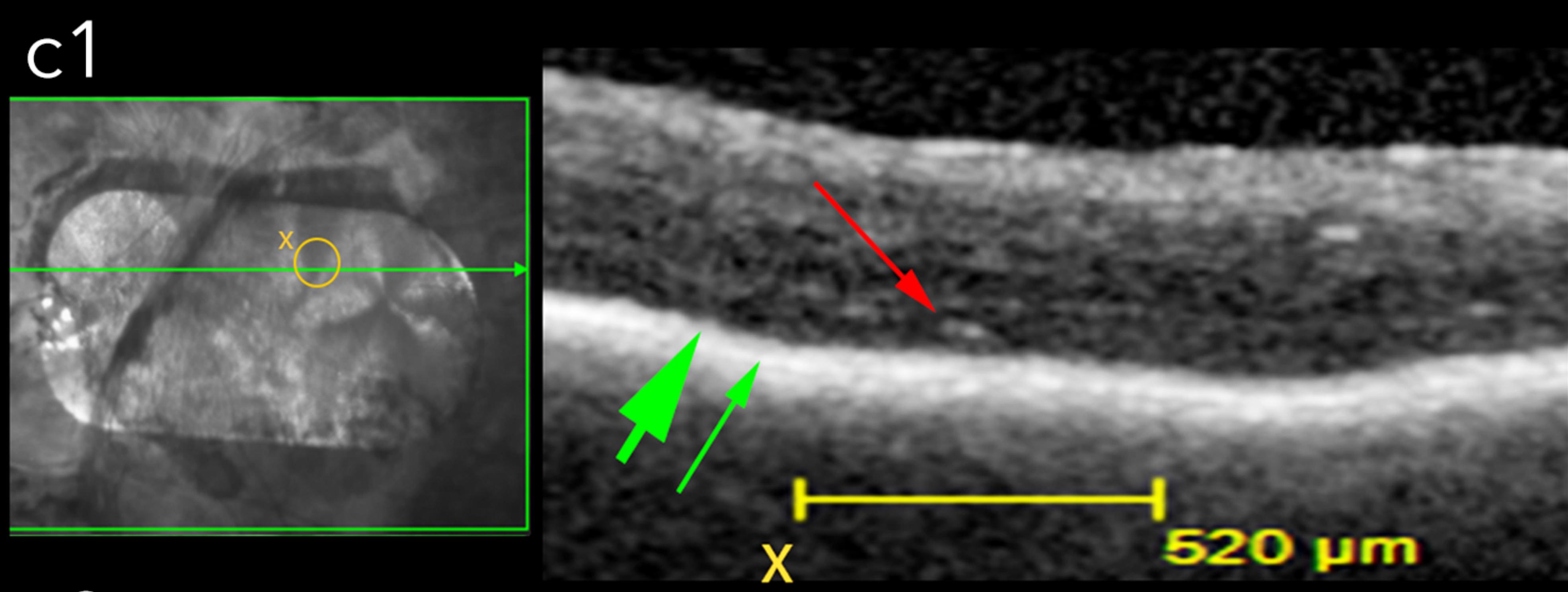
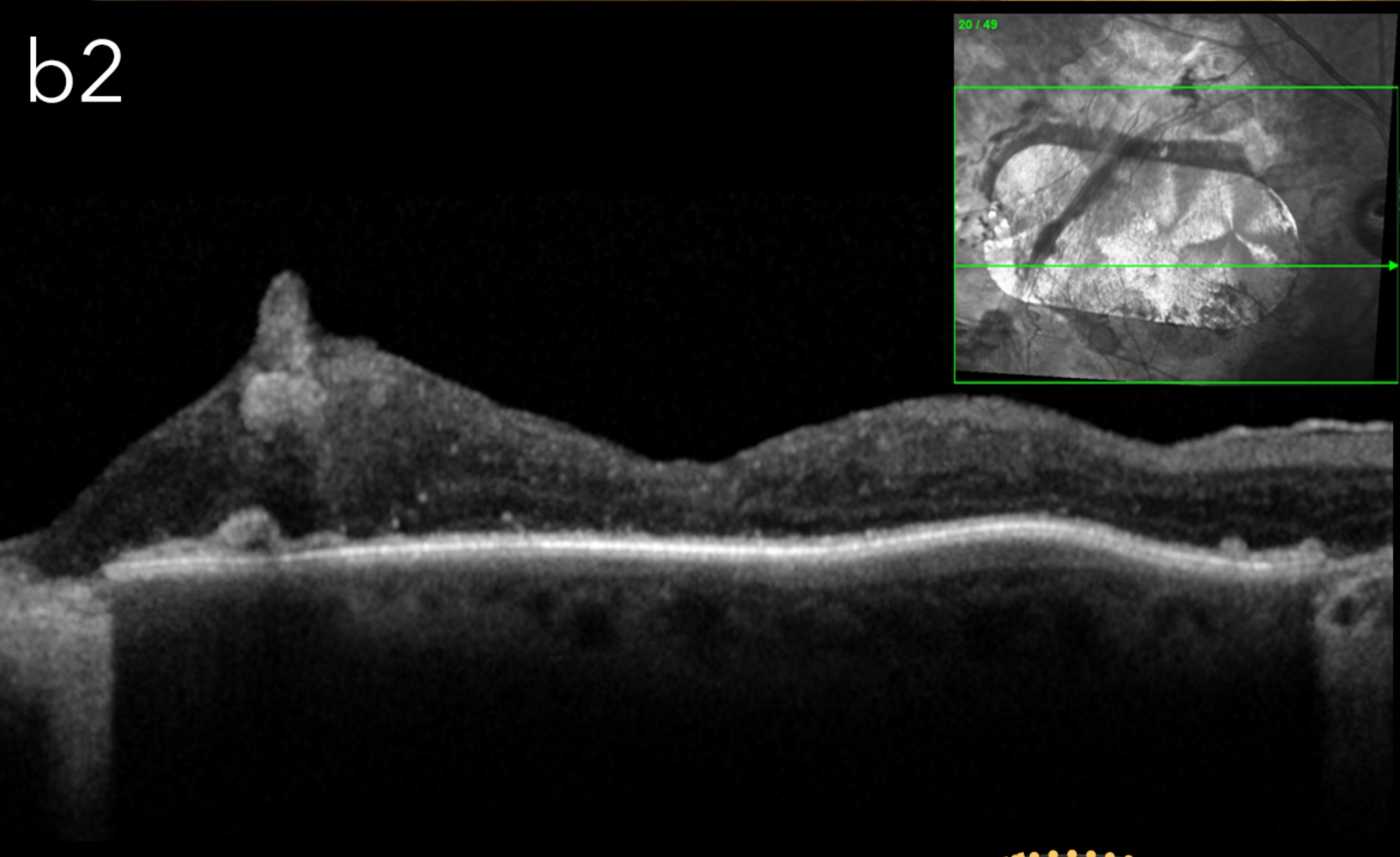
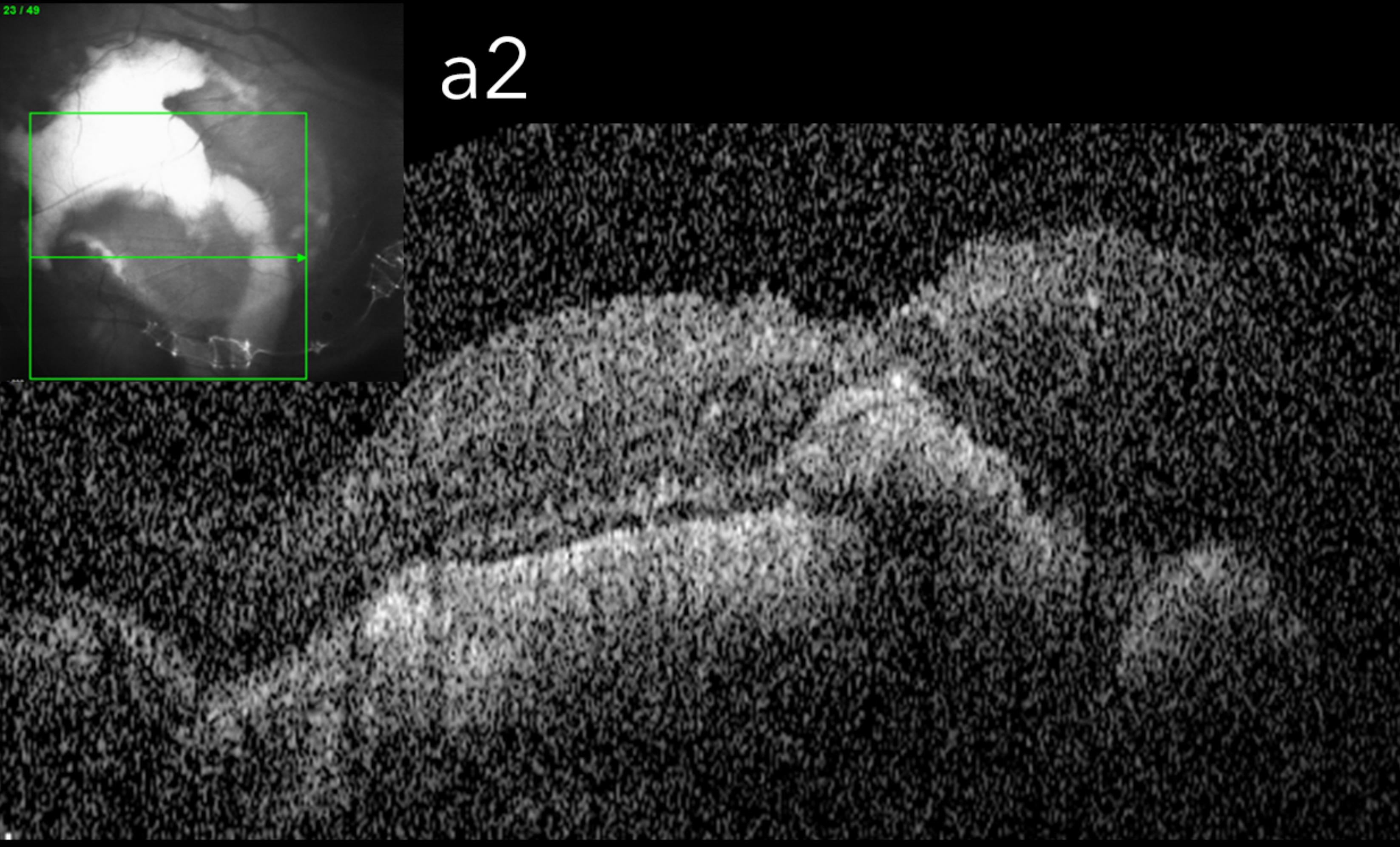
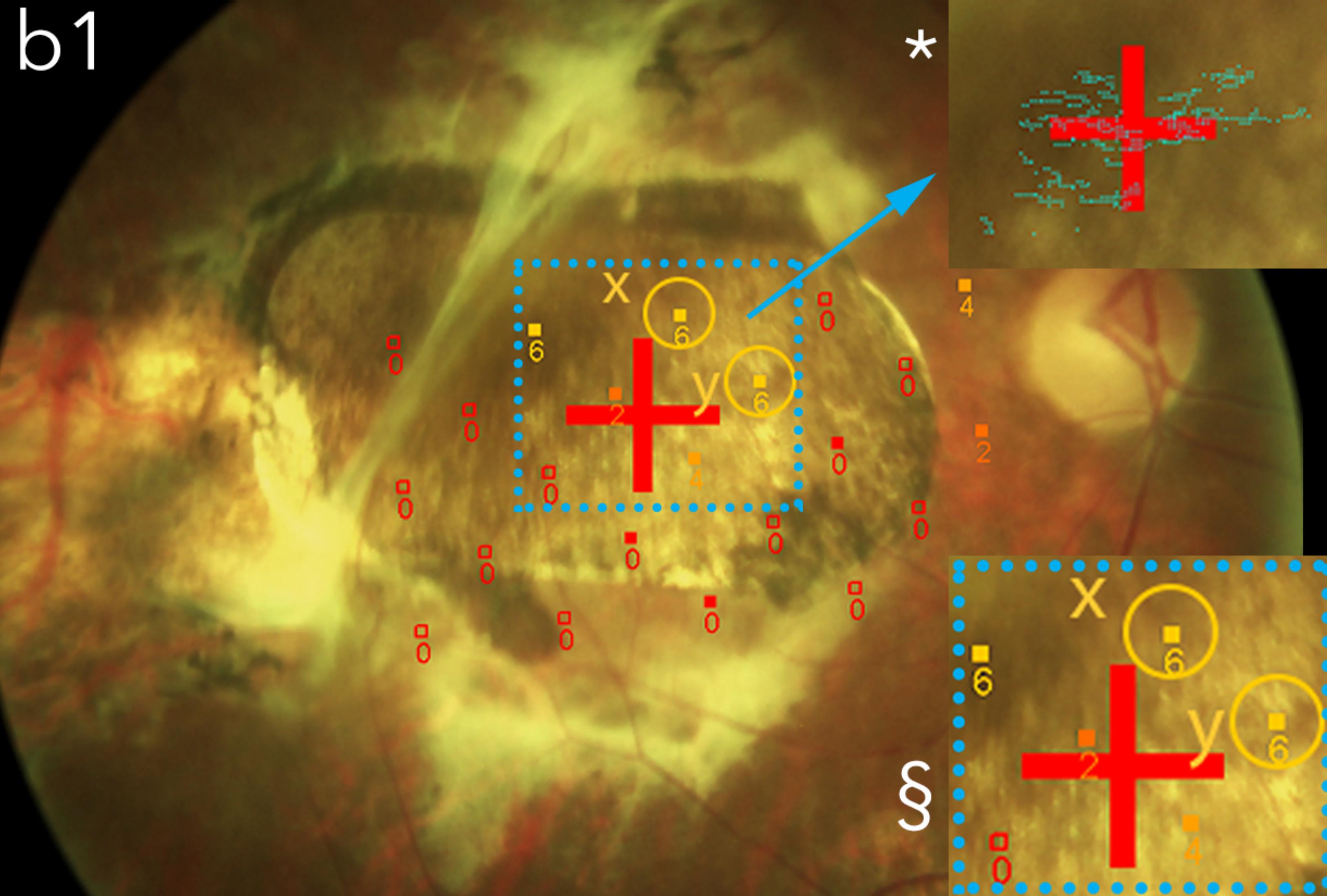
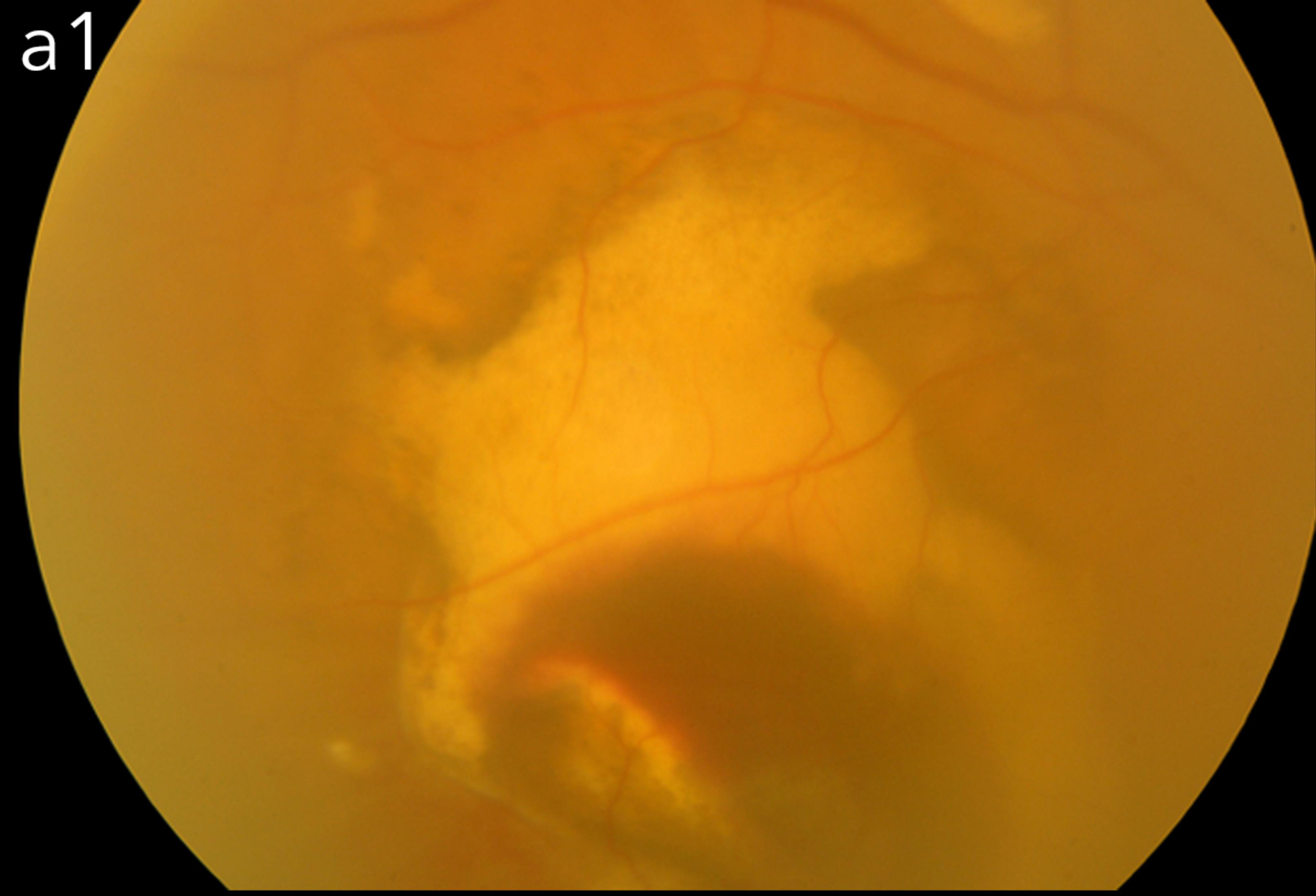
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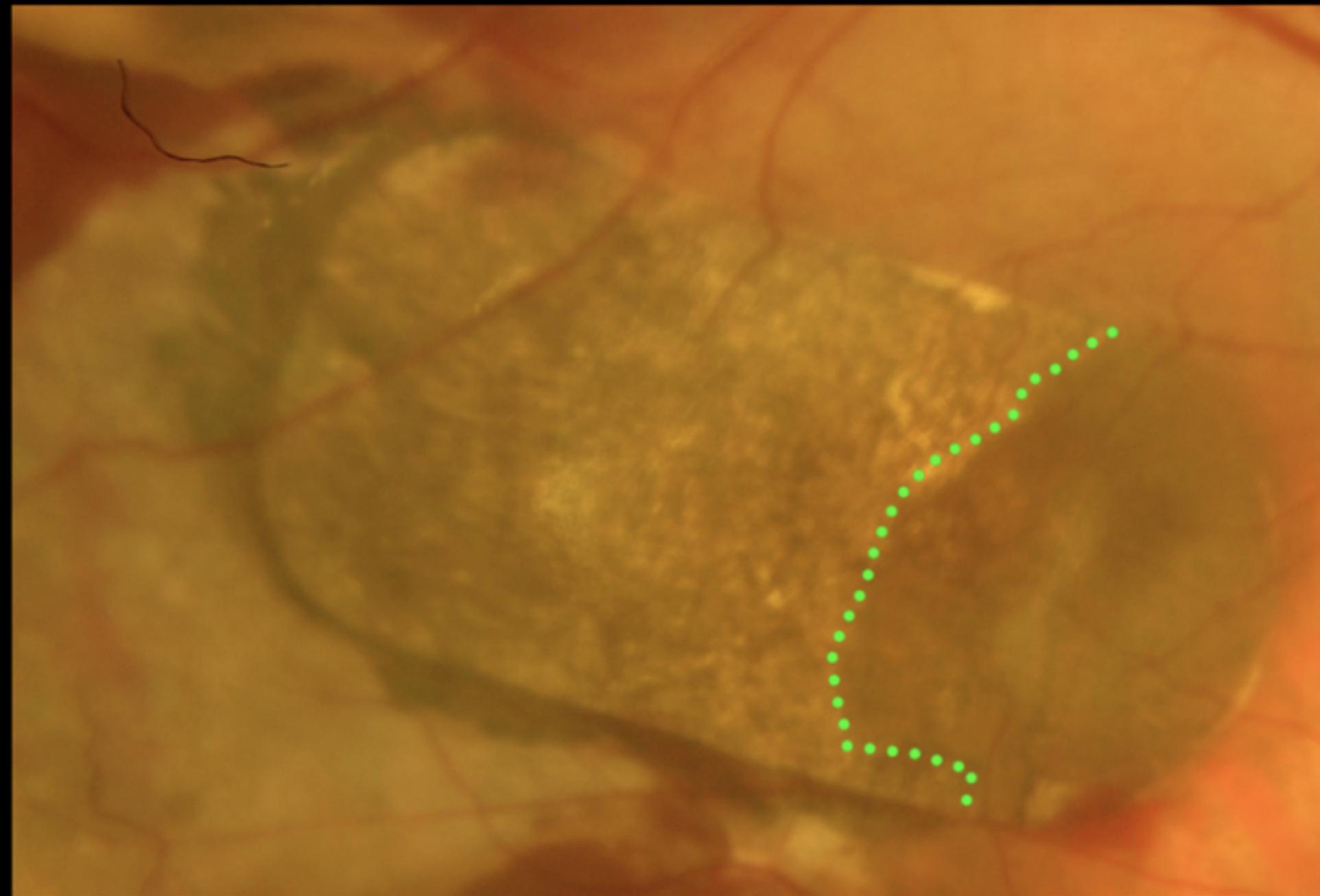


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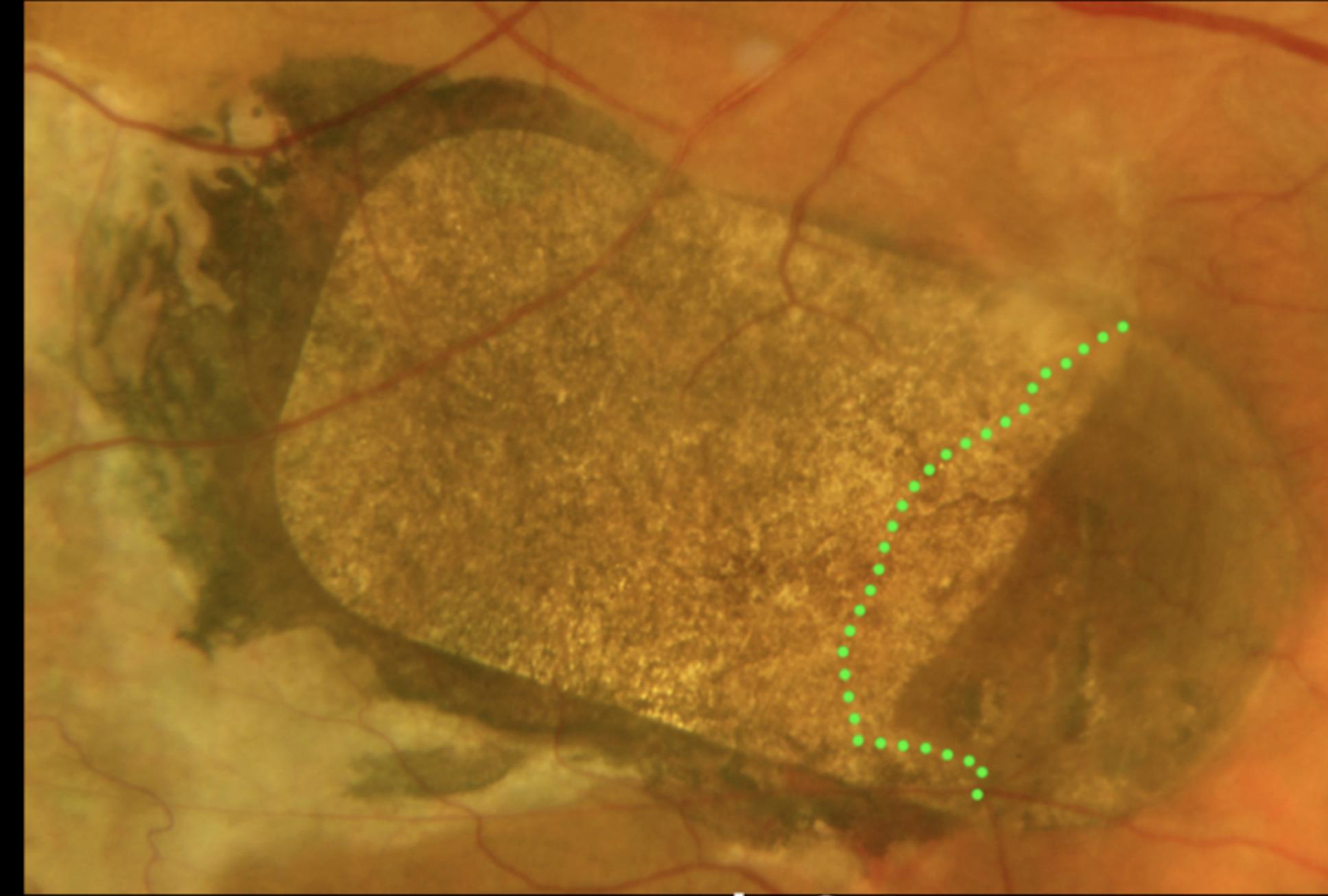




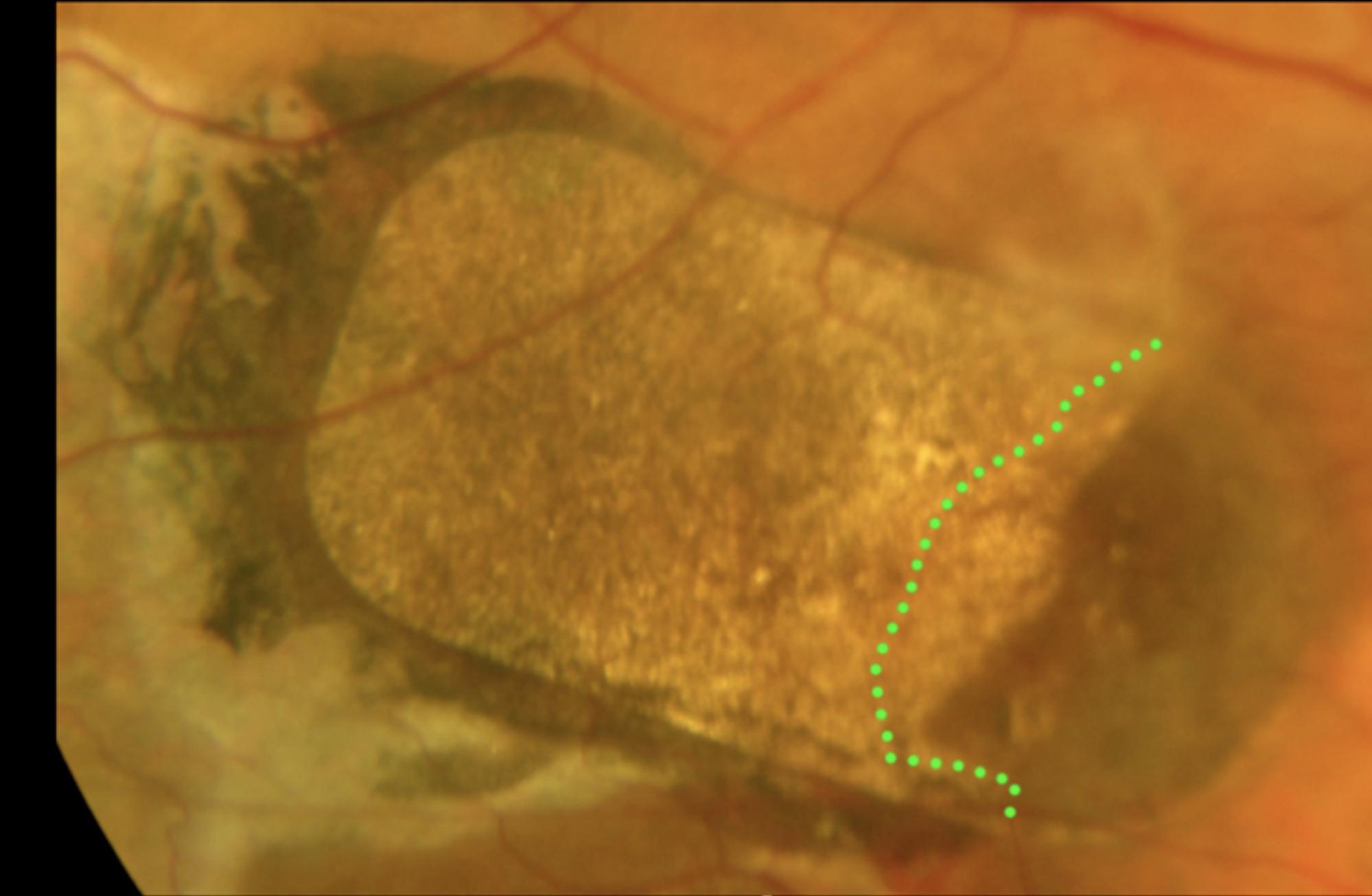
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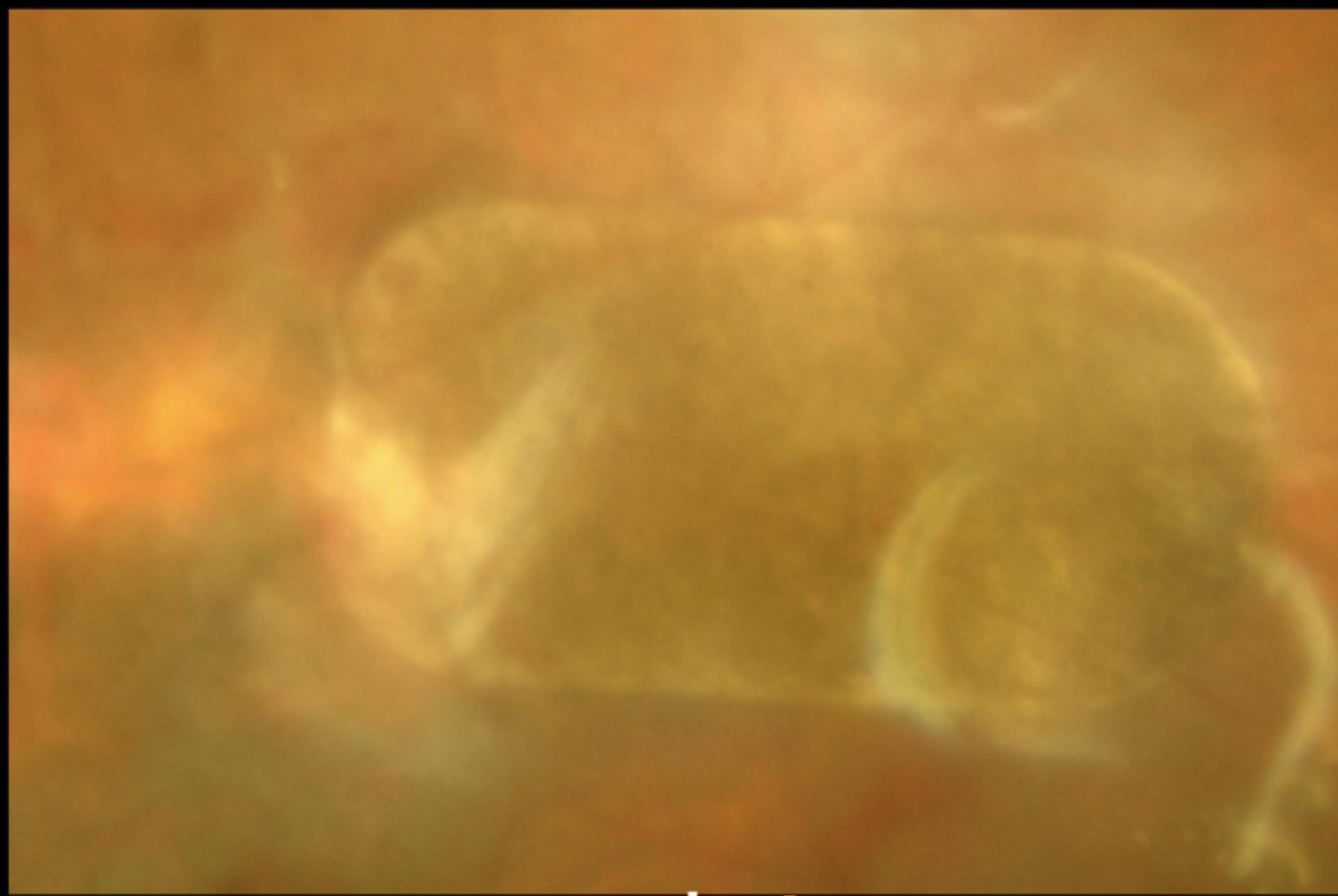


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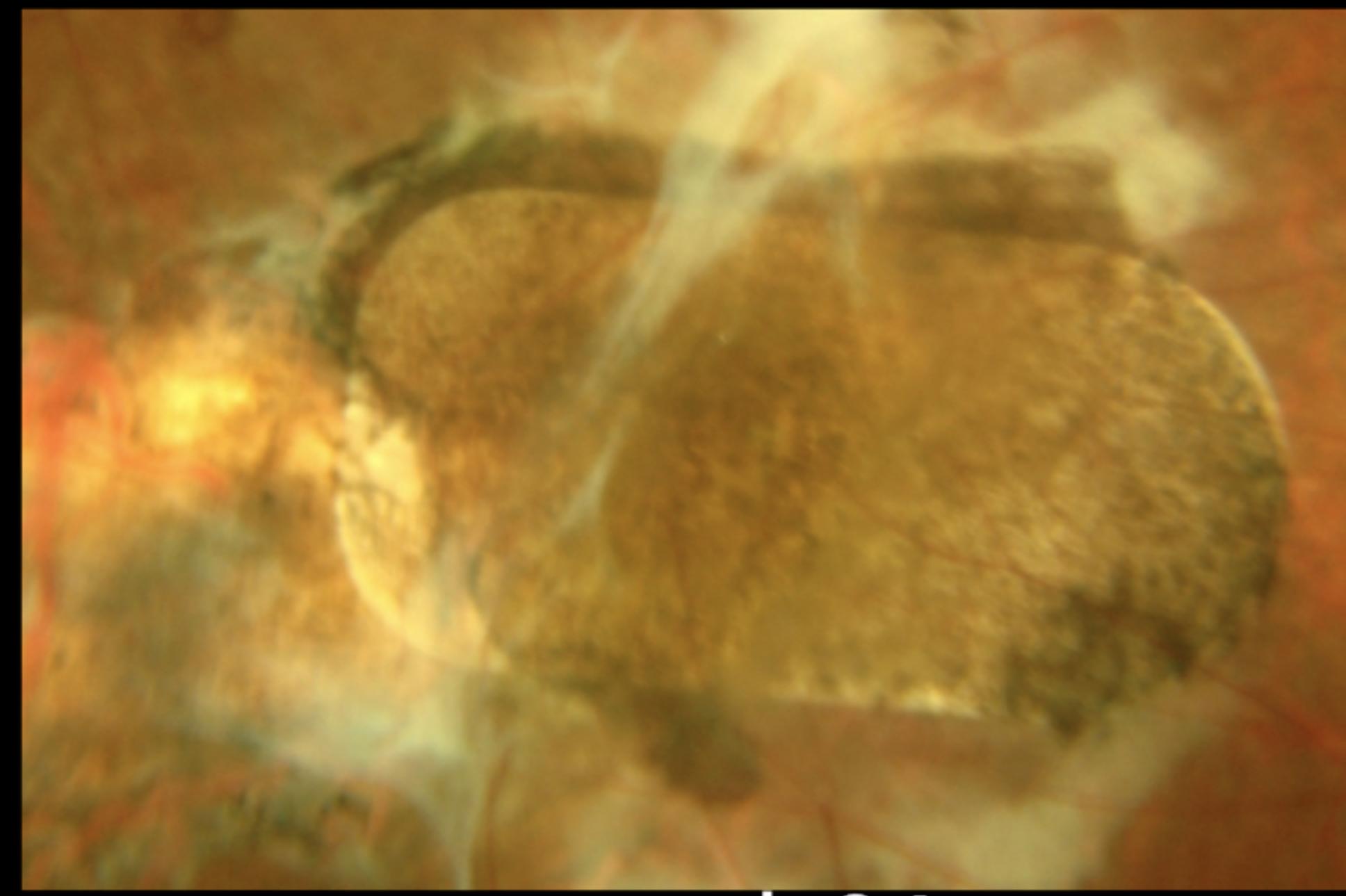


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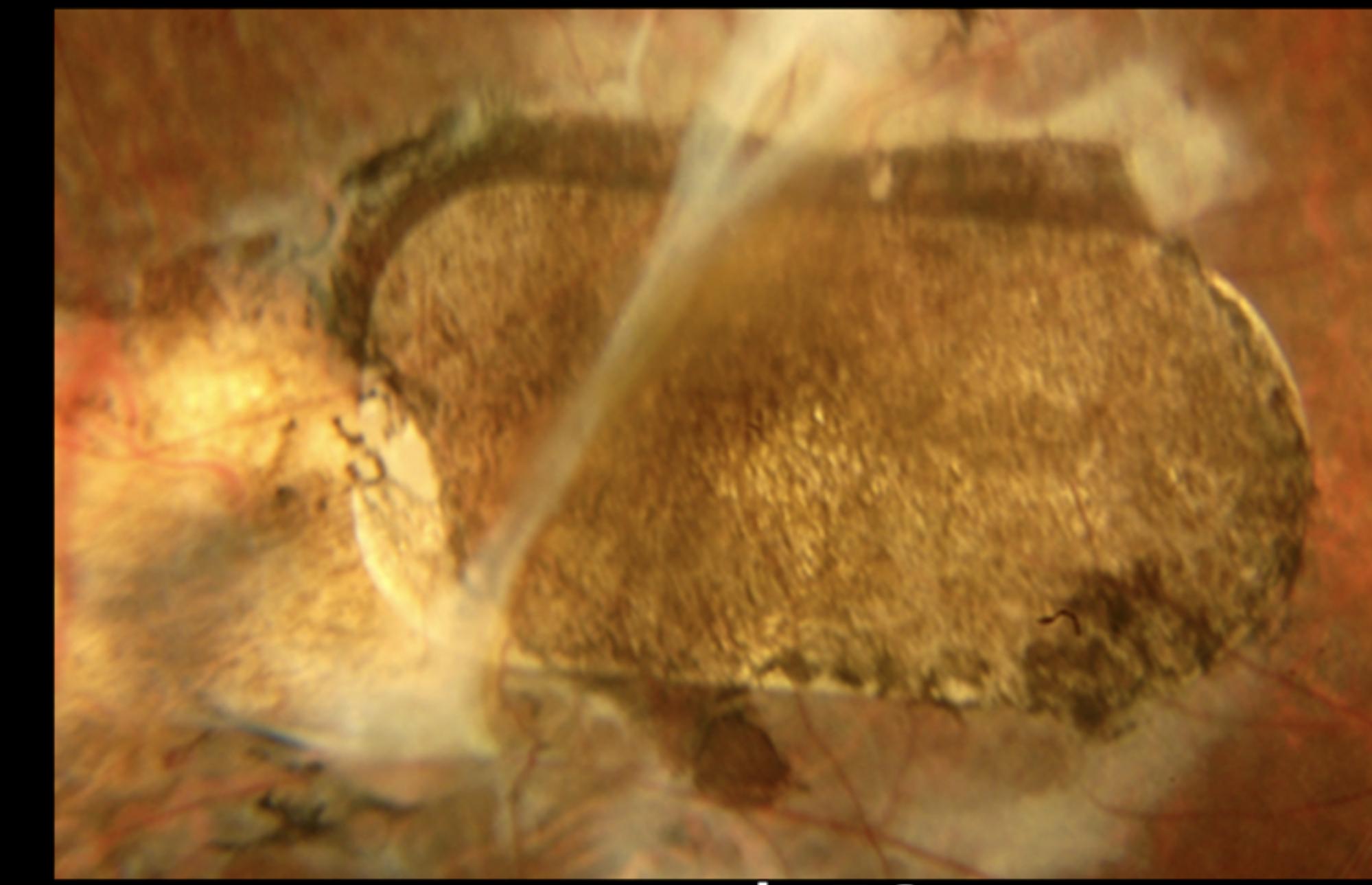
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week 4



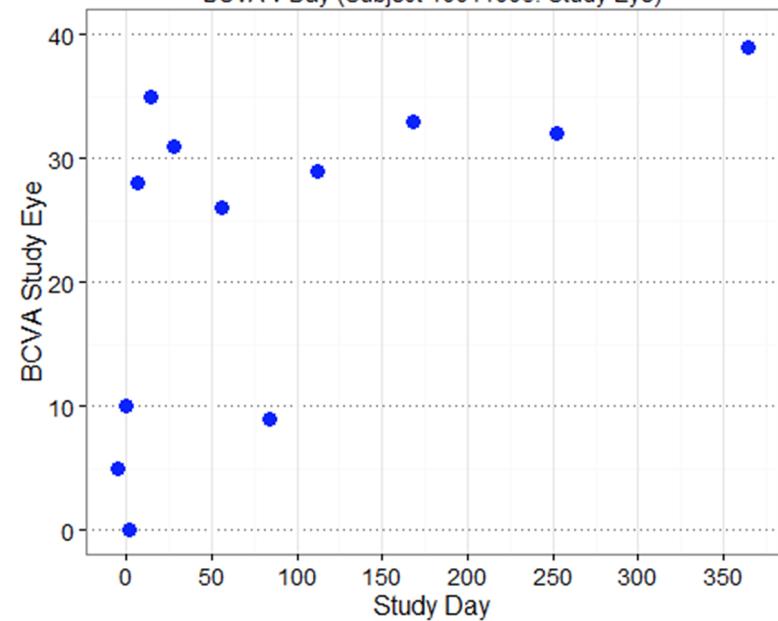
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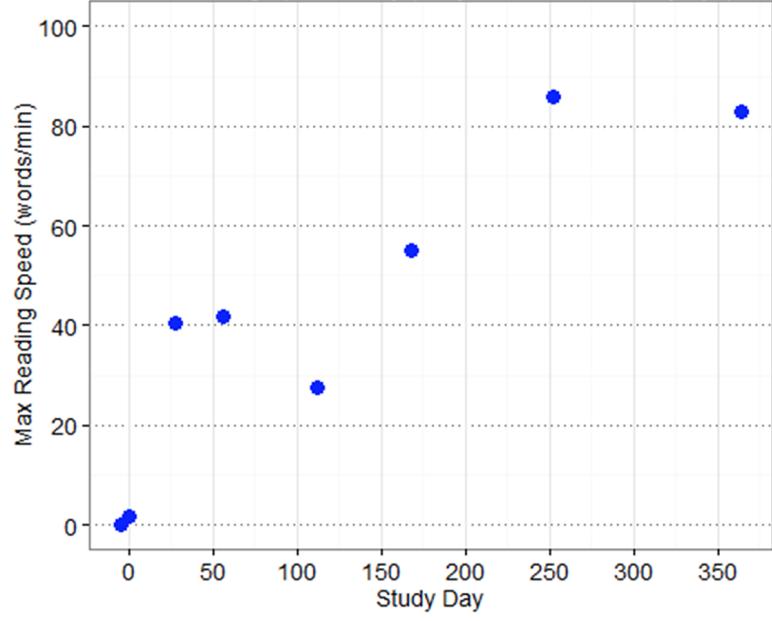
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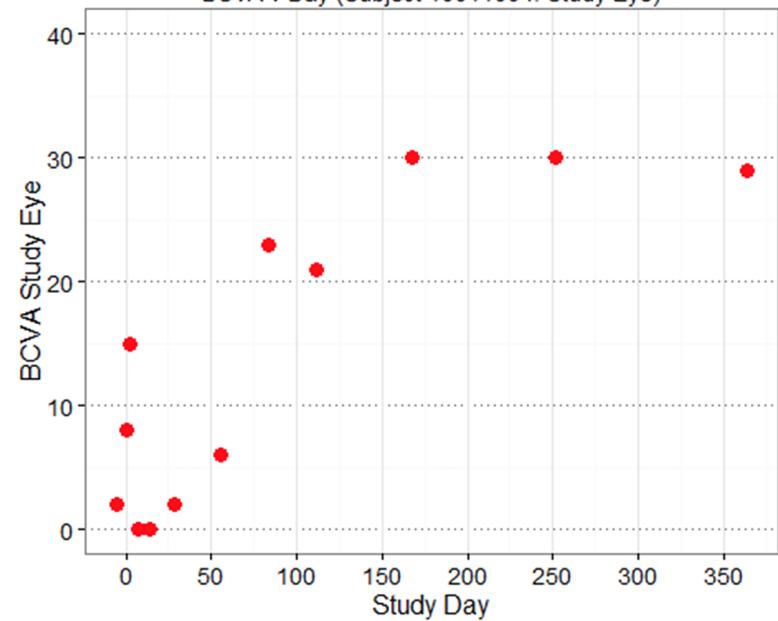
BCVA v Day (Subject 10011003: Study Eye)

**b.**

Max Reading Speed v Day (Subject 10011003: Study Eye)

**c.**

BCVA v Day (Subject 10011004: Study Eye)

**d.**

Max Reading Speed v Day (Subject 10011004: Study Eye)

